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A METHOD OF STUDYING THE MOVEMENT OF RESPIRATORY GASES THROUGH WAXY COATINGS[1]

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(WITH TWO FIGURES)

Introduction

It is commonly assumed, when considering the movement of carbon dioxide and oxygen through waxy coatings applied to fruits and vegetables as a preservative measure, that the movement of water vapor, as measured by loss in weight, is an index of the efficiency of the coating in impeding the movement of all gases. This obviously is not necessarily so, and some direct method of measuring the permeability of such coatings to oxygen and carbon dioxide would be of value in studying the behavior of fruits and vegetables which have been waxed to increase their storage life. This has been accomplished, using a small cucurbit fruit as a test object, by determining the effect of the coating upon the composition of gases in the internal cavity.

Methods and apparatus

Although methods for the micro-analysis of respiratory gases are available, the micro-gas analyzer of BLACET, MACDONALD and LEIGHTON (**1**) being perhaps the best, it was felt that the peculiar difficulties involved in performing rapid successive analyses upon samples of gas withdrawn from the internal cavity of a pumpkin justified the designing of a new type of gas analyzer. In particular, it was desired to be able to draw samples of gas directly into the analyzer without the necessity of an intermediate transfer vessel, with its concomitant delay, disturbance of the fruit, and opportunities for contamination.

This apparatus is shown in figure 1. Unlike that of BLACET *et al.*, it makes free use of stopcocks, but these are so located that the gas which is being analyzed does not pass through them, nor is it possible for grease from them to dirty the burette. This design has the additional advantage of per-

[1] Paper no. 1972, Scientific Journal Series, Minnesota Agricultural Experiment Station.

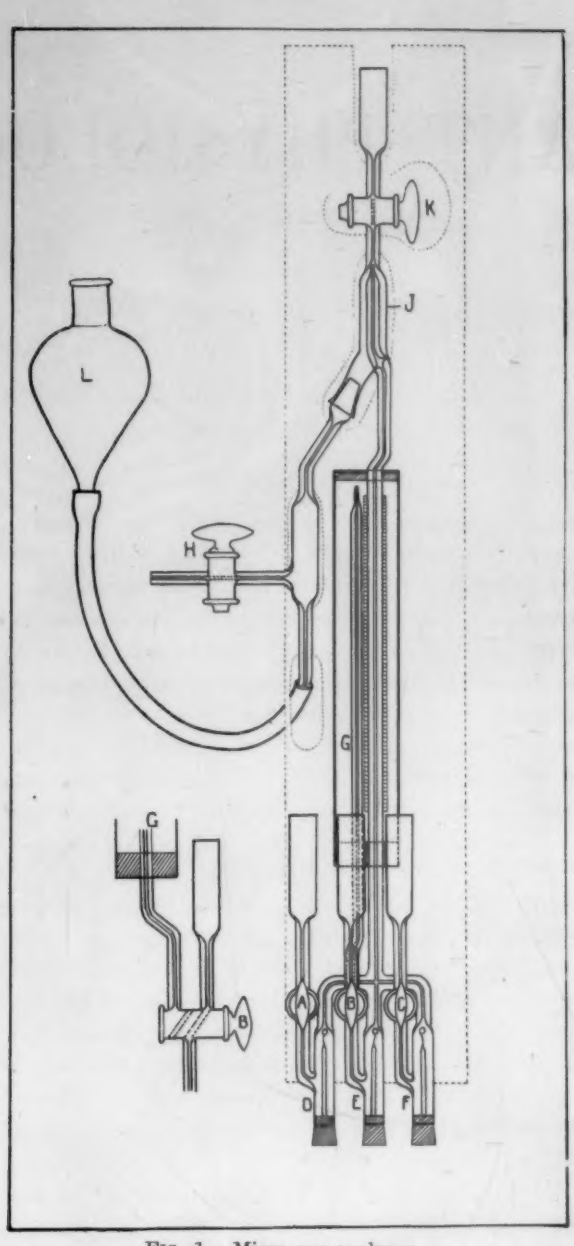

FIG. 1. Micro-gas analyzer.

mitting the use of a compensator tube for adjusting the pressure of the gas in the burette.

Its operation, in principle, is as follows. Gas may be caused to move in

the burette by manipulation of the levelling bulb, L, accompanied by the opening of one of the stopcocks, A, B, or C. By opening the corresponding stopcock, gas may be made to pass into one of the absorbers, D, E, or F, where it is retained until the pressure is so altered as to reverse the flow of the mercury, when it may be returned to the burette for measurement.

At the beginning of an analysis, the whole apparatus, exclusive of the compensator, G, is filled with mercury. By lowering the bulb and opening the cock, H, gas is drawn into the system through the side tube. The side tube is closed, and one of the stopcocks on the absorbers is opened. When the bulb is raised, the gas passes into the chamber, J, and down into the burette. When the proper amount of gas is in the burette, the stopcock is closed and the bulb lifted to a level with the stopcock, K, which is then opened, expelling the excess gas. Then, without lowering the bulb, one of the absorber stopcocks is opened and the gas in the burette is brought into position for measurement, the upper part of the burette filling with mercury. The stopcock, B, is turned to the position which connects the compensator with the burette, the pressure in the burette is adjusted, and the volume of the gas read.

The gas is passed successively into the absorbers by manipulation of the stopcocks and the levelling bulb, the volume being measured after each constituent is absorbed. When the analysis is complete, the residue is expelled through the stopcock, K, and the apparatus is ready for the next determination.

Oxygen is absorbed by a bead of yellow phosphorus held on the end of a platinum wire. The bead is formed by melting phosphorus under water in a suitable mold. Carbon dioxide is absorbed by means of a pellet of sodium hydroxide which is made to adhere to a platinum wire by touching the red hot end of the wire to it, and water is removed by calcium chloride fused on a platinum wire. The phosphorus bead may be used for from three to six determinations, depending upon the quantity of oxygen, after which it may be renewed by washing with water. The sodium hydroxide may be used considerably longer, and the calcium chloride indefinitely. Any one of them may be renewed in one or two minutes without detaching the apparatus from the fruit. A 0.1-ml. sample of gas may easily be dried and analyzed for oxygen and carbon dioxide in fifteen minutes.

Certain precautions must be observed in the construction of the apparatus. The capillary tubing used for the burette must be of uniform diameter, and all the seals in the capillary tubes, especially the four way seal, must be very smooth and regular, with a diameter no larger than that of the capillary. The corks which close the bottom ends of the absorbers must be of exceedingly fine grain, and quite firm, so as not to "give" to any great extent with the changing pressure in the apparatus.

In these experiments a small "squash" or more properly, pumpkin, of

the variety Table Queen was used. Under sterile conditions a hole was cut in the stem end with a corkborer, and a capillary tube closed with a heavy rubber tube and screw clamp was inserted and sealed with vaseline. This treatment did not appear to harm the fruit. The fruits were waxed and immediately attached to the gas analyzer by means of the rubber tube. Before each analysis, a little gas was withdrawn and discarded in order to wash out the connection.

Three types of waxy coatings were tested in the present experiments, together with a heavy coating of paraffin, which presumably is quite impermeable to respiratory gases as well as to water, and was therefore used as a check. These treaments will hereafter be designated as A, B, and C. A was a commercial wax emulsion intended for use on fruits. It was of the dry-bright type and had a hygroscopic gloss-producing substance in its composition. B was a commercial dry-bright wax emulsion intended primarily for use on furniture, but which gave good results on many fruits. C was a very thin layer of spermaceti wax, of about the same thickness as the layers left by the emulsion waxes, but applied without the use of emulsifying agents. At regular intervals, measured from the time at which the coating was applied (not, in the case of the emulsions, from when the fruit was dry) samples of the gas in the internal cavity were withdrawn and analyzed for carbon dioxide and oxygen.

Results

The efficacy of these treatments in preventing water loss from the fruits was tested, and, on the basis of paraffin being 100 per cent. effective, treatment C was 73 per cent. effective, A, 43 per cent., and B, 13 per cent. effective. If the fruits were cut open after several days, it was found that the paraffined fruits and those given treatment A had strong odors such as might be due to anaerobic respiration, while those given treatments B and C had fresh sweet smells.

The results of the analyses of the internal gases are shown graphically in figure 2. It will be seen that in the fruits treated with paraffin, the oxygen had disappeared from the internal cavity within 90 minutes, while the carbon dioxide rose rapidly. In the case of treatment A, the oxygen again is exhausted rapidly, but here, the carbon dioxide does not start to increase until an hour and a half has passed, this being approximately the time required for the wax emulsion to dry on the fruit. With B, again the oxygen falls off rapidly, but in this case, the carbon dioxide increases at once, and after a little, the oxygen concentration rises, until at the end of a day it is up to six per cent., while the carbon dioxide holds at a level about a third above the initial level. Finally, with treatment C, the oxygen falls off rapidly to a certain point, after which it increases almost as rapidly to practically the original level, and the carbon dioxide increases rapidly to a fairly high level, which is maintained.

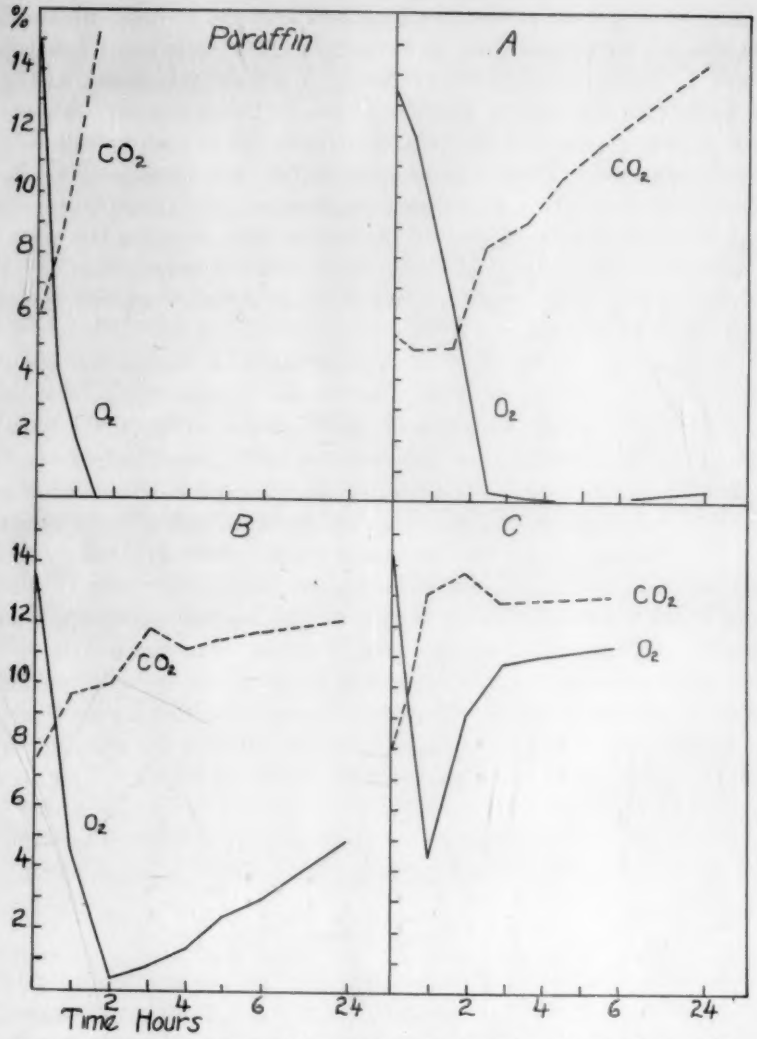

FIG. 2. Changes in composition of internal atmosphere in waxed pumpkins.

Discussion

It need not be further emphasized that these treatments differed radically in their effect upon the composition of the internal atmosphere of the fruit, and that these changes are in no way predictable from the efficacy of the treatments in preventing loss of water.

The results obtained may be explained if it be assumed that a high carbon dioxide content in the fruit was capable of anaesthetizing respiration. On this basis, a wax, in order to be successful in maintaining proper conditions within the fruit, ought to have a relatively low permeability to carbon diox-

ide, in order to permit the concentration of this gas to build up inside the fruit; and a high permeability to oxygen in order to prevent anaerobiosis. The wax C meets this requirement well. With the treatment A, the converse held true, the carbon dioxide produced being readily lost, at least during the early stages of the treatment, and the oxygen consumed being too slowly replaced. Finally the oxygen is gone, not enough carbon dioxide has been accumulated to slow down respiration, and anaerobiosis sets in. Treatment B apparently is just on the border-line, escaping the state of A only by a hair's breadth. It is worthy of mention, however, that of a dozen or so fruits given each treatment, all of those given A spoiled and all of those given B were good.

It is apparent, therefore, that there is much to be learned about the behavior of wax coatings on fruits that cannot be discovered from studies of moisture-loss control. It is to be hoped that studies along the lines indicated will lead to substantial advances in the technique of preserving fruits and vegetables by waxing. The present method ought to be capable of application to any fruit or vegetable having sufficient air space in its interior to permit the withdrawal of a few tenths of a ml. of gas for analysis.

It is also possible to attach a device for extracting gases in a Torricellian vacuum to the analyzer. Using such a device, studies have been made on the effect of coating rutabagas with paraffin on the composition of their internal gases. Rutabagas are coated with a fairly heavy layer of paraffin in commercial practice, this preserving them in good condition for many months. It was found that the oxygen quickly disappears within the root, the carbon dioxide increasing rapidly at first and then falling off slowly. Upon testing, it is found that this tissue produces little or no carbon dioxide under anaerobic conditions, nor is alcohol or any other substance which alters the food value or palatability of the root formed. This matter is worthy of further investigation.

Conclusions

A micro-gas analyzer is described, together with a technique for testing the permeabilities of waxy coatings to respiratory gases, using a cucurbit fruit as a test object. Tests made of three types of waxy coatings indicated that the permeabilities of the coatings to oxygen and carbon dioxide were critical in determining the success of the treatment, the ideal situation in this case being a high permeability to oxygen accompanied by low permeability to carbon dioxide.

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HYDROGEN ION CONCENTRATION IN RELATION TO ABSORPTION OF INORGANIC NUTRIENTS BY HIGHER PLANTS[1]

D. I. Arnon, W. E. Fratzke and C. M. Johnson

(WITH TWO FIGURES)

The findings with regard to the influence of the external hydrogen ion concentration on growth (**2**), particularly the extreme effects observed at pH 3 and 9, have led to a search for a possible explanation of the observed responses in terms of the physiological activities of the plant. A further investigation of the relation of external hydrogen ion concentration to the absorption of inorganic nutrients, which was already elucidated for several ions by Hoagland and Broyer (**5**), seemed particularly pertinent. A series of experiments was therefore initiated to explore the effect of external reaction, within a pH range of 3 to 9, on the absorption of the nutrient ions: Ca^{++}, Mg^{++}, K^{+}, NO_3^{-} and $H_2PO_4^{-}$.

In order to differentiate between the direct effects of external reaction on absorption—which can best be explored by short-time experiments with uniform plants—and the more complex relation of hydrogen ion concentration to growth, a departure was made from the technique followed in the former investigation (**2**). Instead of growing young seedlings for extended periods of time in solutions of varying pH, the general arrangement of the experiments to be presently reported, provided for the use over short periods of time of relatively uniform, healthy plants with well-developed roots, which were grown prior to the absorption experiments in the same complete nutrient solution of a pH favorable to growth.

Methods

Tomato (*Lycopersicum esculentum* variety, Best of All), lettuce (*Lactuca sativa* variety, Imperial 847), and Bermuda grass (*Cynodon dactylon*) were germinated in sand in the greenhouse. Upon reaching a size convenient for transplanting they were transferred to corks in iron tanks (**2**), filled with a complete nutrient solution (table I, no. 1). The plants were kept in the complete nutrient solution for 5 weeks. By allowing the nutrient solution to approach depletion at the end of this period the plants were put in a "low-salt" condition conducive to active absorption (**5**). At the end of this period they were selected for uniformity and placed for 3 days in a "minus-calcium" solution (table I, no. 2). Since no attempt was made to

[1] Acknowledgment is made of clerical assistance given in the preparation of this manuscript by the personnel of the Work Projects Administration, Project #65–1–08–91–B–10.

remove calcium contaminants, however, the "minus-calcium" solution could not be regarded as entirely free from calcium. The healthy appearance of the roots at the time of transfer and their subsequent capacity for absorption indicated that their immersion for 3 days in the "minus-calcium" solution did not result in injury. The purpose of keeping the plants in a "minus-calcium" solution just before the absorption test was to predispose them to calcium absorption. Preliminary experiments indicated that plants failed to absorb calcium at pH 3.

Nutrient solutions nos. 1 and 2 (table I) were each supplemented with 1 ml. of A5 solution (**1**) which supplied 0.5 p.p.m. each of boron and manganese, 0.05 p.p.m. of zinc, 0.02 p.p.m. of copper and 0.01 p.p.m. of molybdenum. Iron was added to nutrient solutions nos. 1 and 2 thrice weekly, at the rate of 0.7 ml./liter of 0.5 per cent. solution of ferrous sulphate.

At the end of the three-day period in nutrient solution no. 2, the plants were transferred for the duration of the absorption period, 96 hours, to individual tanks of about 40-liter capacity filled with nutrient solution no. 3 (table I), supplemented only with 0.5 p.p.m. of boron as H_3BO_3. The reaction of the nutrient solution in each tank was adjusted with either NaOH or H_2SO_4 to one of a series of pH values between pH 3 and 9, in gradations of 1 pH. The control of the initial pH was similar to that previously described (**2**), except that more frequent adjustments, three to six times daily, were resorted to.

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS

	NUMBER 1	NUMBER 2	NUMBER 3
	M.	*M.*	*M.*
KNO_3	0.005	0.005	0.005
$Ca(NO_3)_2$	0.0015	.....	0.0005
$MgSO_4$	0.001	0.001	0.0005
$NH_4H_2PO_4$	0.001	.....	.....
KH_2PO_4	.....	0.00006	0.00006

Na_2SO_4 was added to all solutions below pH 9 in amounts calculated to give the same concentration of sodium in all tanks. The sulphate concentration in the several tanks was allowed to vary, as this was the ion selected for the maintenance of the electro-ionic balance.

The concentrations of calcium, magnesium, potassium, phosphate and nitrate ions were analytically determined on a sample of the nutrient solution no. 3 (table I) at the beginning of the experiment and at the end of each 24-hour period. Potassium was determined by the volumetric cobaltinitrite method of HIBBARD and STOUT (**4**); magnesium, by the 8-hydroxy quinoline method described by SNELL and SNELL (**7**); phosphate, by a modi-

fication of the DENIGES method (**3**); nitrate, colorimetrically by the phenol-disulphonic acid method (**3**); and calcium by the standard oxalate-permanganate titration (**3**). The amounts absorbed were computed by difference and expressed in terms of milliequivalents or milligram-ions absorbed by a single plant. Positive values indicate a net absorption and negative values a loss from the plant to the nutrient solution.

Throughout the absorption period of 96 hours the same number of plants was used with 40 liters of nutrient solution for each pH value: 11 for Bermuda grass, 10 for lettuce, and 6 for tomato. The low phosphate concentration in the nutrient solution during the period of absorption, made it necessary to analyze from time to time for phosphate and to replenish it to its original concentration as needed.

All nutrient solutions were continuously aerated by means of porous carbon tubes (**2**).

Results

Within a short time—one hour or less—after the tomato and lettuce plants had been placed in a nutrient solution at pH 3, a distinct injury to the plants was invariably observed. The roots appeared damaged by showing a dull greyish color and a perceptible loss of turgidity. On clear warm days these symptoms of the roots were associated with prompt wilting of the shoots of the tomato and lettuce plants. Recovery from wilting usually occurred during the first night but the plants wilted again in the morning. Unlike the tomato and lettuce, the injury at pH 3 to the roots of Bermuda grass was relatively slight, and no wilting of the shoots was generally observed.

On the other extreme of the pH range, at pH 9, no visible damage to the plants was noted. The relation between the external hydrogen ion concentration and absorption will be discussed presently but so far as visible injury over short periods of time is concerned it was found to be confined to plants immersed in a nutrient solution at pH 3.

Figure 1 gives the results at the end of 96 hours, of absorption tests on tomato, lettuce, and Bermuda grass. Since an absorption period of 96 hours was conceivably long enough to obscure short time adjustments of plants to an unfavorable external reaction, additional time-absorption data at pH 3 are presented in table II.

An inspection of figure 1 and table II discloses at once the peculiar nature of absorption at pH 3. Common to all three plants at this hydrogen ion concentration is either a total failure or a great reduction in the absorption of calcium and phosphate. In the case of lettuce there is an actual loss of these nutrients from the plant to the nutrient medium. A similar loss was found for the tomato in the early periods of absorption (table II). Owing to the fact however, that these plants were grown for several weeks

prior to the absorption experiment in a complete nutrient medium and hence could conceivably have had some calcium phosphate precipitated on the root surfaces, there is some doubt, particularly if the amounts involved are small, whether this "negative absorption" should be attributed to a withdrawal of calcium and phosphate from the roots or to a dissolving of the surface precipitate. Yet the prompt and profound injury of the lettuce and tomato roots observed at pH 3 render the former interpretation probable.

The injury to the roots of Bermuda grass at pH 3 in the absorption experiment was relatively slight, and no loss, in fact a gain, of phosphate

TABLE II

ABSORPTION AT pH 3.
MILLIEQUIVALENTS PER PLANT ABSORBED AT THE END OF EACH PERIOD

		HOURS			
		24	48	72	96
Ca	Tomato	−0.33	0	0	0
	Lettuce	−0.28	−1.14	−0.85	−0.28
	Bermuda grass	0.20	0.20	0.20	.....
PO_4*	Tomato	−0.08	−0.01	−0.01	0.03
	Lettuce	−0.03	−0.05	−0.08	−0.10
	Bermuda grass	0.02	0.06	0.18	0.29
Mg	Tomato	−0.38	1.15	0.82	0
	Lettuce	−0.19	0	0.42	0.33
	Bermuda grass	0.16	0.30	0.50	0.86
K	Tomato	0.17	1.35	−0.17	−0.51
	Lettuce	−1.31	1.75	−0.44	−0.43
	Bermuda grass	−0.41	0.92	2.25	3.38
NO_3	Tomato	2.87	2.77	5.23	4.70
	Lettuce	−1.38	−1.19	−1.29	1.01
	Bermuda grass	0.71	4.52	7.87	10.70

* Computations were made on the assumption that phosphate was absorbed as $H_2PO_4^-$.

was noted (table II). But the marked restriction in phosphate absorption at pH 3, in comparison with absorption at higher pH values, was apparent (fig. 2). With respect to calcium, however, Bermuda grass behaved more like tomato and lettuce: no further absorption occurred after the first 24 hours. In fact a loss of calcium at the end of 96 hours is indicated. It will be recalled in this connection that as with the other plants no growth of Bermuda grass took place at pH 3 (**2**). The conclusion then, that at pH 3 there is serious interference with the absorption of calcium and phosphate, seems warranted by all the evidence at hand.

As far as the other ions at pH 3 are concerned, Bermuda grass absorbed appreciable amounts of magnesium, potassium and nitrate (fig. 1, table II). The one negative value for the absorption of potassium by Bermuda grass at the end of the first 24 hours (table II), is followed by increasing absorp-

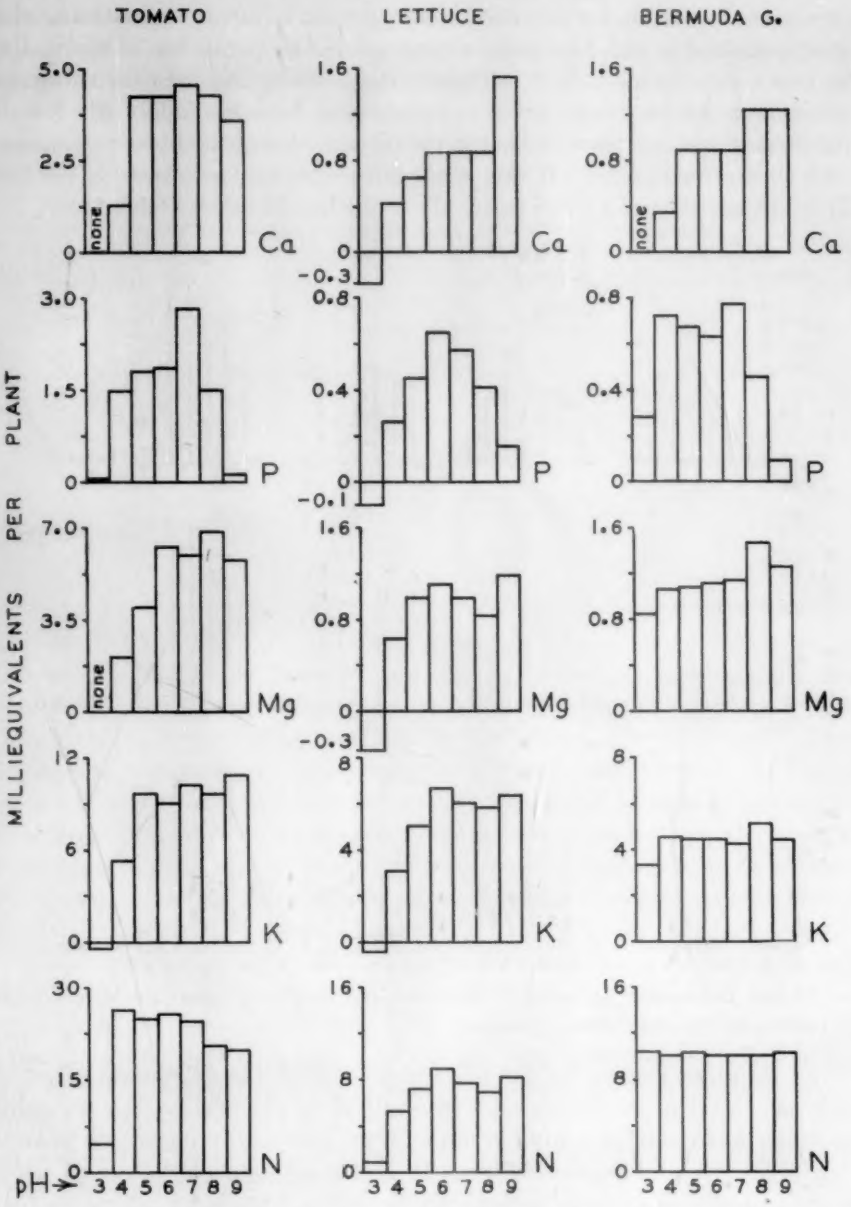

FIG. 1. Effect of pH on absorption of inorganic nutrients at the end of 96 hours by tomato, lettuce, and Bermuda grass. Phosphorus was assumed to have been absorbed as $H_2PO_4^-$.

tion at the end of each successive 24-hour period. Tomato and lettuce, however, exhibited at pH 3 no gain in magnesium and potassium at the end of 96 hours, despite indications of intermittent absorption. As for nitrate, a distinction can be drawn between tomato and lettuce. While the tomato plants absorbed measurable amounts of nitrate, though small by comparison with absorption at other pH values, the lettuce plants lost nitrate in the first 24 hours and showed a small gain only in the last 24 hours (table II).

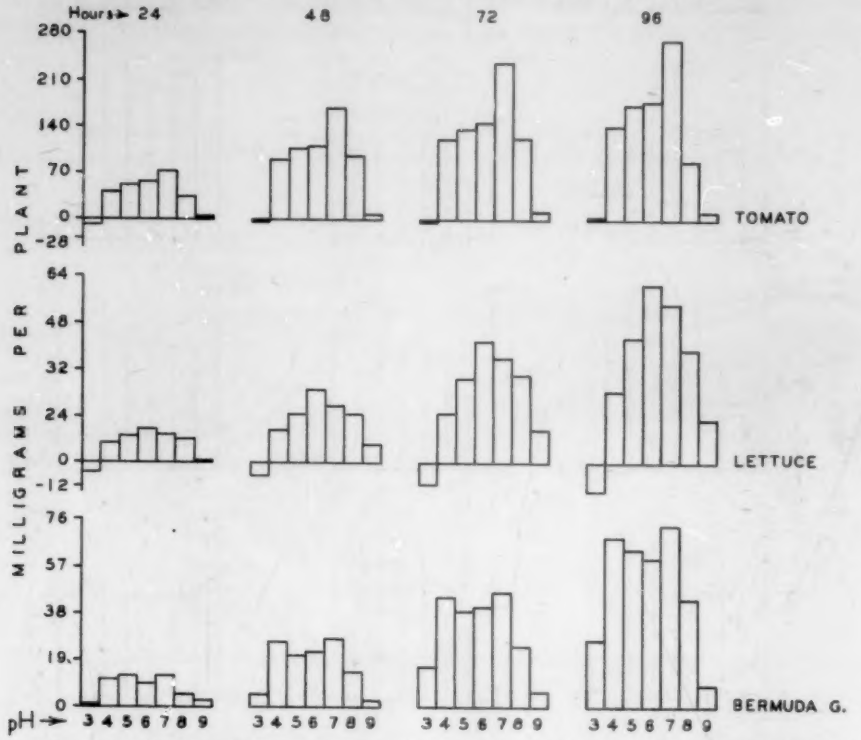

FIG. 2. Influence of pH of nutrient solution and time on the absorption of phosphate by tomato, lettuce, and Bermuda grass.

At the other extreme of the pH scale, at pH 9, the deleterious effect of external reaction on absorption was confined to phosphate. As indicated by the detailed data presented in figure 2, the decrease in phosphate absorption which became apparent above pH 7, was unmistakably clear at pH 9 at the end of each 24-hour period. Unlike pH 3 there is no indication that pH 9 represented a reaction unfavorable for the absorption of calcium, nor of the other nutrients investigated: nitrate, potassium, or magnesium (fig. 1).

The absorption of calcium from nutrient solutions maintained at pH

values between 4 and 8 is of special interest. It was previously reported that at pH 4 and 5 increasing the concentration of calcium in the nutrient solution was conducive to increased growth of tomato and lettuce but that at pH 6 a higher calcium concentration in the nutrient medium was no longer associated with improved growth (**2**). The data given in table III

TABLE III

ABSORPTION OF Ca WITH TIME FROM ACID NUTRIENT SOLUTIONS. MILLIGRAMS PER PLANT ABSORBED AT THE END OF EACH PERIOD

	External pH	Hours			
		24	48	72	96
		mg.	*mg.*	*mg.*	*mg.*
Lettuce	3	−5.7	−22.8	−17.1	−5.7
	4	0	−4.4	0	8.8
	5	0	−4.4	0	13.2
	6	4.4	4.4	13.2	17.6
Tomato	3	−6.6	0	0	0
	4	0	0	13.2	26.4
	5	13.2	33.0	59.4	85.6
	6	19.8	39.6	66.0	85.6

suggest that this effect of calcium on growth is related to the absorption of this element from acid nutrient solutions. Leaving aside the already discussed negative absorption at pH 3, relatively favorable absorption of calcium obtained only at pH 6, particularly for lettuce. At pH 4 and 5 a net absorption of calcium was indicated only at the end of 96 hours. At earlier intervals no calcium was absorbed. Although these relations were not as pronounced for tomato, yet for this plant as well as for lettuce, a decreasing acidity up to pH 6 was associated with an increasing absorption of calcium. A further reduction in hydrogen ion concentration, including pH 9, had no particular effect on calcium absorption.

A decreased absorption of calcium by Bermuda grass, similar to that of tomato and lettuce, was found only at pH 4 (fig. 1). At pH 5 and above no relation between the external hydrogen ion concentration and calcium absorption was indicated. Bermuda grass grew very well at pH 4 and 5 and showed no response at these reactions to an increased calcium concentration in the nutrient solution.

The absorption of nitrate, potassium, and magnesium between pH 5 and 9 was unaffected in any significant degree. At pH 4, however, a difference again was noted between lettuce and tomato on the one hand and Bermuda grass on the other. While there was no indication of a decreased absorption of these three nutrients at pH 4 by Bermuda grass this generally occurred for lettuce and tomato (fig. 1). It will be recalled in this connection

that the growth of lettuce and tomato, but not that of Bermuda grass, was distinctly reduced at pH 4 (**2**).

Discussion

The results of this investigation offer a point of departure in interpreting the previously reported (**2**) effects of hydrogen ion concentration on growth. Of the five ions studied, the absorption of calcium and phosphate at various hydrogen ion concentrations emerges as particularly relevant to the observed growth responses. At pH 3 all three plants—tomato, lettuce, and Bermuda grass—consistently showed a serious curtailment in phosphate absorption and a complete stoppage, if not an actual reversal, of calcium absorption. Regardless of the particular mechanism of root injury which eventuated in these effects, they can in themselves account for the total failure of growth observed at pH 3. Likewise, at the other extreme of the pH scale, at pH 9, the marked reduction in the absorption of phosphate by all plants, can serve as an explanation for the reduced growth at this reaction. It was observed incidentally that tomato plants grown continuously at pH 9 exhibited symptoms of distress not unlike those encountered in phosphorus deficient plants.

The importance of calcium absorption for certain plants when grown at acid reactions is also illustrated by the data presented in table III. The previously observed poor growth of lettuce and tomato at pH 4 and 5 in nutrient solutions low in calcium (**2**), seems to be related to the reduced absorption of this nutrient at relatively high hydrogen ion concentration. At pH 6 and above large absorption of calcium was common to all plants. Whether the maxima in calcium absorption found at the most alkaline reactions for lettuce and Bermuda grass, but not for tomato (fig. 1), have any significance, cannot be answered on the basis of the present data.

Of special interest is the relation of external reaction to the absorption of phosphate; two distinct minima in the absorption of this nutrient were found at pH 3 and at pH 9. Had it been possible to prevent the intermittent shifts downward of the external reaction at pH 9 and to maintain it within as narrow limits as that at pH 3, it seems certain that an even lower phosphate absorption at pH 9 than that actually found could be demonstrated. In any event the influence of external reaction on phosphate absorption at both pH 9 and 3, was unmistakable (fig. 2).

These findings are not entirely consistent with the views of McGeorge (**6**) that phosphate can be absorbed by plants only in the form of $H_2PO_4^-$. It is certainly true that at pH 9 it is impossible to have much $H_2PO_4^-$ in solution since the predominant ionic form of phosphate at this reaction is $HPO_4^=$. $HPO_4^=$, however, is also the predominant phosphate ion at pH 8, and even at pH 7 its concentration exceeds that of HPO_4^-, without causing a drastic reduction in absorption.

But since the external pH determines the ionic dissociation of phosphate there is no opportunity to subject to an experimental test the relative absorption of different ionic forms of phosphate at varying hydrogen ion concentrations. Evidence on the effect of calcium and magnesium concentration in relation to phosphate absorption at high alkalinity and a better understanding of the influence of hydroxyl ions on phosphate absorption, promise to be of interest in this connection.

A distinct contrast, particularly for lettuce and tomato, between nutrient absorption at pH 3 and pH 9, is apparent. Whereas, at pH 9 only the absorption of phosphate was hindered, at pH 3, in addition to the already discussed failure of calcium and phosphate absorption, that of magnesium, potassium, and nitrate was also adversely affected. The question arises as to what extent these latter effects are related to the lack of absorption, or even to an actual loss, of calcium by the plant at pH 3. The influence of calcium on the permeability of cytoplasmic membranes and the absorption and retention of ions (**8**) and the rôle of calcium pectate as a structural component of the cell wall, are pertinent to the question. It should be noted that Bermuda grass, which unlike the other plants showed no initial loss of calcium at pH 3 (table II), was also the plant capable of absorbing other nutrients at this reaction. It is of course probable that even Bermuda grass, in view of its failure to grow at pH 3 (**2**), would cease to absorb with longer exposure to this reaction.

Our results on the effect of pH of the nutrient solution in relation to the absorption of Mg, K and NO_3 are in harmony with the data of HOAGLAND and BROYER (**5**), who have investigated the absorption of potassium, nitrate, and halide within a pH range of around 4 to 8. Their conclusion that within this range of hydrogen ion concentration there is no profound effect of pH on the absorption of these ions is borne out. This concordance of results is noteworthy in view of the differences in experimental technique: excised barley roots used by HOAGLAND and BROYER in an absorption experiment which lasted 9 hours as compared here with entire tomato, lettuce, and Bermuda grass plants studied over a period of 96 hours. The relatively lower absorption at pH 4 in our experiments can be attributed to possible injury as a result of longer exposure. The bearing that evidence of this kind has on suggested theories of absorption has been dealt with extensively by the previous authors.

Summary

As a part of a general investigation of the effects of hydrogen ion concentration on growth of higher plants, experiments were made on the relation of external pH to the absorption of calcium, magnesium, potassium, nitrate, and phosphate.

Five-week-old tomato, lettuce and Bermuda grass plants previously grown under favorable conditions, were placed for 96 hours in a series of nutrient solutions ranging, in gradations of 1 pH unit, from pH 3 to pH 9.

Injury to roots from the reaction of the medium was apparent only at pH 3. At this reaction, no absorption, in fact suggestions of loss, of calcium and phosphate, were in evidence. For tomato and lettuce this reaction was also wholly unsuited for the absorption of other ions.

With the exception of phosphate absorption which showed a marked decrease at pH 9, no other untoward effects were found at this reaction.

Evidence was obtained of lower calcium absorption, particularly by tomato and lettuce, from strongly acid solutions (pH 4 and 5) than at higher pH values.

No profound effects of external reaction between pH 4 and 9 on the absorption of magnesium, potassium, and nitrate were noted.

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INFLUENCE OF HYDROGEN ION CONCENTRATION ON THE GROWTH OF HIGHER PLANTS UNDER CONTROLLED CONDITIONS[1,2]

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(WITH FOUR FIGURES)

The effects of hydrogen ion concentration on plant growth occupy a prominent position in discussions of the adaptability of plants to their nutrient environment. In the last two decades, with the improvements of methods of measuring pH and particularly with the widespread adoption of the glass electrode, increasing attention has been given to the hydrogen ion concentration in soils as it may influence their suitability for different crop plants.

An understanding of the responses of plants to soil reaction is dependent: first, on the determination of the true pH of the soil; and secondly, on a knowledge of the effects of hydrogen ion concentration on plant growth. There is considerable doubt at present about the precise meaning of a soil pH measurement obtained by standard procedures including the glass electrode.[3] But even if some particular pH value were to be assigned to a soil as truly representative of the hydrogen ion concentration that happens to characterize the boundary between the soil and the plant root, there still would remain the difficulty of interpreting a soil pH value in terms of an isolated and independent variable. Is the failure of plants to thrive in an acid soil due to a high hydrogen ion concentration or to such other unfavorable factors of which a low pH is generally symptomatic; for example, a depletion of calcium and the presence of toxic amounts of aluminum or manganese in the soil solution? On the other extreme, there is the question whether poor plant growth in a soil characterized by a relatively high pH is to be attributed to a high hydroxyl ion concentration or to the unavailability of such plant nutrients as phosphate, iron, and manganese.

In addition to these indirect effects associated with pH which make it difficult to interpret the responses of plants grown in a soil, there is a dearth of information about the effects of hydrogen ion concentration on plant growth, with other factors of the nutrient environment held constant. The purpose

[1] The substance of this paper was presented before the American Society of Plant Physiologists, at the A.A.A.S. meetings at Philadelphia, December 1940.

[2] Acknowledgment is made of clerical assistance given in the preparation of this manuscript by Work Projects Administration, Project #65–1–08–91–B–10.

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of this investigation was to secure evidence which would permit the evaluation of such direct effects of hydrogen ion concentration on the growth of higher plants. Emphasis was placed on the development of an experimental technique which would make it possible to isolate hydrogen ion concentration as a single variable, over a range of pH from 3 to 9, in gradations of 1 pH unit. For the reasons just cited the use of soil was considered unsuited to the main objective. A water culture technique suitably manipulated afforded an opportunity to control satisfactorily the ionic concentrations in the nutrient medium, including that of hydrogen ion.

Methods

The pH range from 3 to 9 was selected for study. The inclusion of alkaline reactions within the pH range raised at once the question of precipitation of nutrient ions on the alkaline side of neutrality and the subsequent alteration in the composition of the nutrient solutions with respect to ions other than hydrogen. An illustration of such indirect effects of pH on growth through an alteration of the composition of a nutrient solution is afforded by the work of REED and HAAS with calcium at alkaline reactions (**9**). In order to prevent changes in the nutrient solution because of precipitation the procedure developed in an earlier investigation (**1**), of adopting for the entire range of pH the concentration of the various ions that can be maintained at *the highest pH* investigated, was used throughout.

A standard nutrient solution was prepared and its pH adjusted to 9 by the addition of NaOH. The resulting precipitate was removed by filtering and the filtrate was analyzed for Ca, Mg, K, NO_3 and PO_4. Analysis of the filtrate served as a guide for the composition of the basic nutrient solution used in this study as given in table I. This basic nutrient solution, subse-

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS

	BASIC	LOW CALCIUM	HIGH CALCIUM
	M.	*M.*	*M.*
KH_2PO_4	0.00006	0.00006	0.00006
K_2SO_4	0.0015	.....	0.0065
KNO_3	0.010	0.013	.....
$Ca(NO_3)_2$	0.002	0.0005	0.007
$MgSO_4$	0.001	0.001	0.001

quently adjusted with either NaOH or H_2SO_4 to the various pH values, was used in all experiments unless otherwise indicated.

In order to avoid precipitation at pH 9, it was necessary to reduce drastically the concentration of phosphate ion. As the plants grew, the

solutions were regularly tested for phosphate and additions up to the original concentration were made as needed.

In several experiments varying amounts of calcium were supplied to plants grown at pH 3, 4, 5 and 6. The three solutions, low calcium, medium, and high calcium furnished 20, 80 and 280 p.p.m. of calcium, respectively.

Sulphate was the only ion, other than hydrogen and hydroxyl, whose concentration varied in the pH as well as in the calcium series. Since the variations in the concentration of the sulphate ion in the respective nutrient solutions were regarded as of comparatively small physiological significance, this ion was selected for maintaining the balance of the electro-ionic charges.

The adjustment of the alkaline reaction entailed the addition of NaOH. In order to compensate for this introduction of sodium, Na_2SO_4 was added to nutrient solutions in amounts computed to furnish as much sodium as was added in the form of NaOH to the solution maintained at pH 9; that is, the nutrient solution which required the largest amount of base.

A special problem demanding attention was that of supplying the heavy metals iron and manganese. As the inorganic salts of these are insoluble in alkaline solutions it was decided to furnish these metals in the form of humates which remain soluble over the pH range 3 to 9 and are not precipitated by phosphate (**7**). A synthetic potassium humate solution was prepared from sucrose according to the procedure of HORNER, BURK, and HOOVER (**7**). Stock solutions of humate iron and manganese were made by adding to the potassium humate solution a solution of each metal as a sulphate to constitute 10 per cent. by weight of the humate. The pH of the resulting humate metal solution was adjusted to around 7.

Two mg. each of manganese and iron as the respective humate metals were added to a liter of nutrient solution. No further additions of either iron or manganese were made during the growth of the plants except when the entire nutrient solution was changed.

No deliberate addition of zinc, copper, or molybdenum was made to the nutrient medium. The absence of deficiency symptoms of these micronutrients justified the reliance which was placed on the impurities contained in the ordinary distilled water and salts used (**10**). As these metal impurities were free to combine with the humate accompanying the iron and manganese, no other provision was made to insure their availability throughout the pH range. 0.5 p.p.m. of boron was furnished to all cultures as H_3BO_3.

In order to provide a wide basis in arriving at conclusions on effects of hydrogen ion concentration on the growth of higher plants three different species were included in this study: tomato (*Lycopersicum esculentum*), lettuce (*Lactuca sativa*), and Bermuda grass (*Cynodon dactylon*). The Marglobe variety of tomato and the Imperial 847 variety of lettuce were used. Several experiments were carried out with each species but the responses of the tomato plant were investigated most intensively.

The seed was germinated on moist cheesecloth which was suspended on a Pyrex glass tray filled with redistilled water. When the seedlings attained a height of several inches they were transferred to previously described (1) iron tanks of 115 liters capacity. From six to ten (generally ten) plants, each supported in a cork, were placed in a tank and grown for a period of 5 weeks in the experimental nutrient solutions.

The fresh weights were usually expressed as averages of the ten plants grown in each tank. For fresh weight determinations the shoots were weighed directly and the roots were centrifuged before being weighed (5).

As pointed out in a previous paper (1), it is well to consider, in studying the effects of hydrogen ion concentration, the reaction of the culture medium in the zone directly adjacent to the absorbing root surfaces. These are in a dynamic equilibrium with the immediately adjacent zone of the culture medium and continuously alter its composition and reaction. That the reaction in this zone may be different from that a short distance away from the roots was shown by NIGHTINGALE (8).

The use of a suitable stirring device to bring about a continuous circulation of the nutrient solution within the tank is a material aid in the removal of such gradients. In the experiments under consideration the aeration technique served not only as a means of maintaining a high oxygen tension around the root but also as an effective stirring device, continuously mixing the nutrient solution and thus contributing to the maintenance of a uniform reaction and composition.

Porous carbon tubes, $\frac{3}{4}$ inch in diameter, extending through the entire length of the tanks, were used as aerators. They were connected by means of rubber tubing to an air line of 10 pounds pressure. A glass rod was placed inside each aerator to prevent the latter from floating. Two aerators provided each tank with a rapid stream of fine air bubbles without causing undue agitation of the nutrient solution.

The hydraulic jack and screw press method of expressing sap from frozen plant tissues (5) was followed. A pressure of 2000 lb. per square inch was used.

All pH measurements whether of the nutrient solution or of the expressed plant sap were made with the glass electrode.

The experimental technique provided for the following general devices in the prevention of large shifts from the original reaction of the culture medium: (a) Large volume of nutrient solutions in relation to the number of plants—between 10 to 20 liters to a plant. (b) Weekly changes of the nutrient solution as the plants grew older. (c) Continuous circulation of the solution within the tank brought about by the rapid bubbling of air. Changes in reaction and composition of the culture medium brought about by the metabolic activities of the plants were thus distributed over a large volume of solution and rendered quantitatively less pronounced.

The maintenance of the original pH was also aided by a continuous-drop device which consisted of a 500-ml. volumetric flask inverted in a 150-ml. beaker, both being supported on a ring stand above the tank. The flask and the beaker were filled with either NaOH or H_2SO_4 of suitable concentration (N/10 or N/100), and a measured amount was delivered into the respective tanks by means of a bent capillary tube, one end of which was supported by a cork in the beaker and the other delivering the acid or base to the tank, through a funnel inserted in a cork in the cover of the tank. The rate of delivery was adjusted by raising or lowering the level of the liquid in the beaker with reference to the tip of the delivery end of the capillary tube. To insure rapid mixing the cork supporting the funnel through which the acid or base was being introduced into the tank, was placed in the cover of the tank directly over an aerator.

An attempt was made to maintain the initial pH of the nutrient solution with fluctuations not exceeding $\pm$ 0.2 pH. The combination of the control devices used was more than equal to maintaining the initial pH of the acid nutrient solutions within these limits. At the less acid reactions, however, and particularly at pH 8 and 9, the plants, especially as they grew larger toward the end of the five-week period, shifted the initial reaction beyond the limits indicated. The pH in each tank was determined twice daily by means of the glass electrode and when necessary adjusted with NaOH or H_2SO_4.

Results

The general plan of the investigation afforded an opportunity to test the validity of the observed responses not only by using different species of plants but also by repeating a given experiment with one plant at various seasons of the year. The principal conclusions of this study as illustrated by the data presented, are based on magnitudes far exceeding those assignable to errors of variability in the plants used.

Effect of external pH on growth

With all plants at all times a complete failure of growth occurred at pH 3. The roots of the seedlings were unable to grow in the nutrient solution maintained at this hydrogen ion concentration, and collapsed soon after their immersion. In some cases as illustrated by that of the Bermuda grass (fig. 3), if the level of the nutrient solution was allowed to drop somewhat in the tank, a tuft of roots developed *above* the solution. In no case, however, was root growth observed in the nutrient solution kept at pH 3. Such limited growth of shoots at pH 3 as is shown in figure 3 was probably occasioned by a restricted absorption by the roots above the level of the nutrient solution which in turn was made possible by the fine spray produced by the

aerators or perhaps by the wick-like absorption of the injured roots in the solution or by both.

On the other extreme, injury and a marked reduction in growth were observed in plants grown at pH 9 (table II and figs. 1, 2, 3). In no case, however, was it as pronounced as at pH 3. Of the plants used, the relatively best growth was made by Bermuda grass. But even this plant which is commonly noted for its resistance to alkali injury was incapable of normal growth in a nutrient solution maintained at pH 9.

TABLE II

EFFECT OF THE pH OF THE NUTRIENT SOLUTION ON GROWTH

External pH	Average fresh weight in grams					
	Tomato		Lettuce		Bermuda grass	
	Shoots	Roots	Shoots	Roots	Shoots	Roots
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
3	.....	.....	.....	.....	8.6	2.9
4	22.3	13.0	85.1	26.0	83.0	24.3
5	83.2	23.5	285.4	50.8	88.5	20.3
6	92.7	19.1	292.6	44.7	104.9	14.8
7	80.9	19.4	162.8	28.1	102.2	15.7
8	49.1	15.4	165.1	36.5	72.1	10.3
9	4.3	2.7	8.2	5.3	20.7	4.9

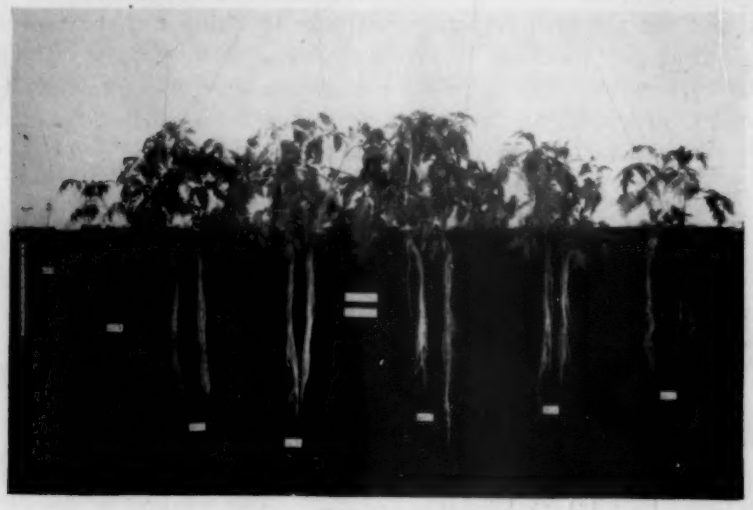

FIG. 1. Effect of external pH on the growth of tomato. From left to right: pH 3, 4, 5, 6, 7, 8, and 9.

Unlike the plants grown at pH 3, some root growth, particularly in the case of Bermuda grass, was observed in the nutrient solution proper at pH 9. Above the level of the nutrient solution healthy appearing roots were in evidence. As for the shoots, a general reduction in growth was common to all plants grown at pH 9. In the case of the tomato plants a purpling on the under side of leaves, characteristic of phosphate deficiency was observed at that pH. This observation is interesting in the light of the data on absorption of phosphate at pH 9 (**3**).

Definite growth of shoots and roots was obtained with all plants at pH 4,

FIG. 2. Effect of external pH on the growth of lettuce. From left to right: pH 3, 4, 5, 6, 7, 8, and 9.

but with the exception of Bermuda grass, it was distinctly restricted (table II, figs. 1 and 2). Bermuda grass at pH 4 grew very well, fully on a par with plants at higher pH values (table II, fig. 3). This was confirmed in several experiments carried out at different seasons of the year.

Good growth with all plants was obtained in the range of pH above 4 and below 8. Some curtailment of growth was noticeable at pH 8; there is little doubt, however, that within this range of pH, variations in hydrogen ion concentration *per se* are associated with no drastic consequences to the growth of the plant. The general suitability of this relatively wide range of pH between 4 and 8 for the growth of higher plants warrants more emphasis than the fluctuations in growth reflected in table II. Although the data presented here as well as other experiments not included in this report suggested a possible "optimum pH" at around 6, it is indeed questionable

whether this was not merely a reflection of a particular ionic proportion in the nutrient solution. Changing the relationships between ions is, as will be shown presently in the case of calcium, a factor in determining the response of plants in certain ranges of hydrogen ion concentration.

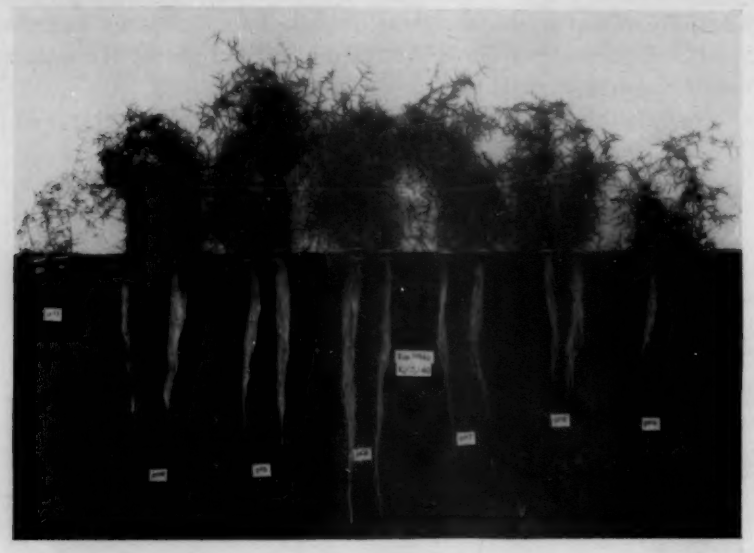

FIG. 3. Effect of external pH on the growth of Bermuda grass. From left to right: pH 3, 4, 5, 6, 7, 8, and 9.

EFFECT OF CALCIUM ON THE GROWTH OF PLANTS IN AN ACID MEDIUM

Results from a parallel investigation (**3**) have suggested that at least one of the causes of toxicity of an acid reaction is to be sought in the interference of a high hydrogen ion concentration with the absorption of calcium from a low level of supply. Experiments were set up, therefore, to compare the growth of plants in nutrient solutions ranging from pH 3 to 6 with three levels of calcium: 20, 80 and 280 p.p.m. The basic nutrient solution as given in table I furnished 80 p.p.m. of calcium and the "low-" and "high-calcium" solutions supplied 20 and 280 p.p.m., respectively. Since the large volumes of the regularly changed nutrient solution in relation to the number of plants insured against the depletion of calcium at the lower concentrations, it is justifiable to interpret the responses noted below, in terms of true concentration differences. The results for lettuce and tomato are presented in figure 4.

No effect of the higher calcium concentration was noted at pH 3. The influence of hydrogen ion concentration at that pH dominated at all three calcium concentrations and complete failure of growth resulted. A dis-

tinctly favorable effect of increased calcium supply was noted, however, at pH 4. With lettuce, progressively improved growth was obtained as the calcium concentration increased to 280 p.p.m., whereas with the tomato the marked increase in growth occurred upon increasing the calcium concentration from 20 to 80 p.p.m. A further increase to 280 p.p.m. was not associated with a large increase in growth. The noteworthy fact, however, for both kinds of plants at pH 4, was the pronounced favorable response to an increase in the concentration of calcium in the nutrient medium. The best

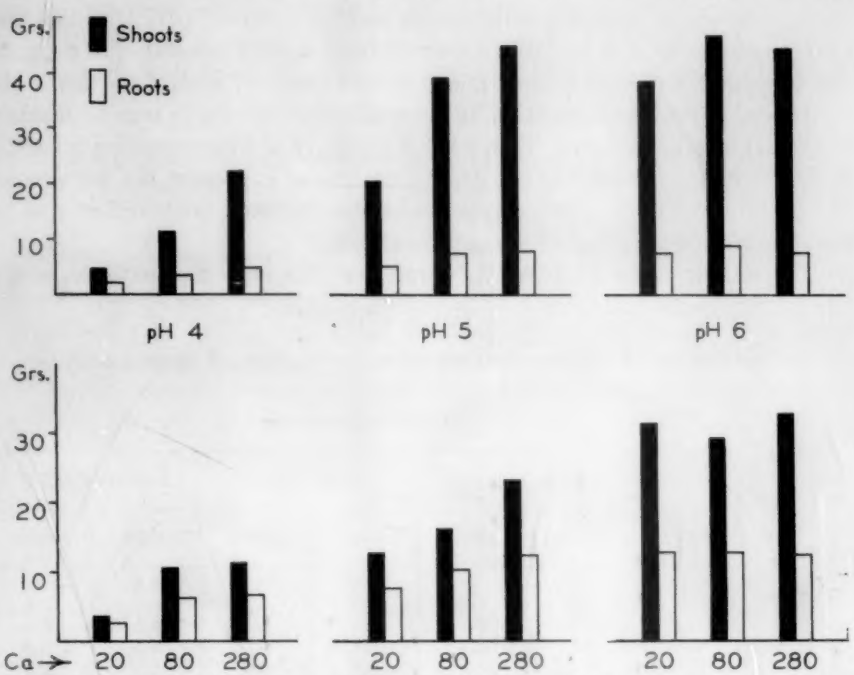

FIG. 4. Effect of calcium concentration on the growth of acid reactions of letture (above) and tomato (below). Fresh weight given in grams.

plants at pH 4 (plants grown with the highest supply of calcium) made as much growth as the poorest plants at pH 5—plants grown with the lowest supply of calcium.

The favorable effect of a higher calcium concentration on growth was also observed at pH 5 for both lettuce and tomato. At pH 6, however, no such response from increasing the calcium supply was found with either species; it will be noted, however, that at this pH, about equally good growth was obtained with all three levels of calcium supply. The best plants at pH 5, *i.e.*, those supplied with 280 p.p.m. of calcium, made approximately as much growth as the plants grown at pH 6.

Effect of external hydrogen ion concentration on the pH of expressed sap

A point frequently raised in discussions of hydrogen ion effects on plants is one relating to changes in the pH of cellular fluids as a result of variations in the external reaction. The pH values of the expressed sap of plants grown throughout the range of hydrogen ion concentration considered in this investigation are given in table III.

It is recognized that these data fail to throw light on possible variations in the reaction of individual tissues, caused by external pH, of which the epidermis and the cortex of the root would be of special interest. In expressing the sap of a root or a shoot a composite sample of several tissues is obtained and a possible fluctuation in the reaction of one tissue may be masked by the pH of other tissues. Despite this difficulty of interpretation inherent in the method, however, the pH determinations of expressed sap were made to determine whether some correlation existed between large differences in the external pH and that of the expressed sap.

The results given in table III show that the wide fluctuations in the

TABLE III

Effect of external hydrogen ion concentration on the pH of expressed sap

pH of nutrient solution	pH of expressed sap						
	Tomato			Lettuce		Bermuda grass	
	Leaves	Stems	Roots	Shoots	Roots	Shoots	Roots
3	.....	.....	.....	.....	.....	5.8	.....
4	6.1	6.1	6.6	6.1	6.1	6.0	5.6
5	6.2	6.1	6.4	6.1	6.2	6.1	6.0
6	6.1	6.1	6.3	6.1	6.0	6.1	6.0
7	6.0	6.1	6.2	6.0	6.0	6.1	6.2
8	6.1	5.8	6.0	6.0	6.2	6.0	6.3
9	6.1	5.8	6.4	6.1	6.3	6.0	6.7

hydrogen ion concentration of the nutrient solution have not been reflected in the pH of the expressed sap. The relative constancy of the reaction of the sap of the aerial portions of the plant was particularly consistent but this was also true of the roots, with the exception of Bermuda grass. With this grass there was found at both extremes of the external pH a corresponding shift in the pH of the root sap. These fluctuations, however, were small relative to the respective changes in the external hydrogen ion concentration and are regarded as not materially detracting from the principal conclusion: namely, that the reaction of the sap is relatively unaffected by shifts in the pH of the nutrient medium.

At pH 3 no root growth occurred and obviously no pH measurement on the sap was possible. In one case, however, enough sap for a pH determination was expressed from the previously mentioned tufts of root which grew above the level of the solution maintained at pH 3 and its pH was found only 0.1 lower than that of sap of roots grown at pH 4.

Effect of Ca on the pH of plant sap

It was deemed desirable to determine whether the favorable effect of calcium on growth at acid reactions (fig. 4) was associated with any changes in the pH of the expressed sap. As shown by the results of the pH determinations assembled in table IV, the increase of calcium concentration in the

TABLE IV

Effect of calcium on the pH of expressed sap of plants grown in acid solutions

pH of nutrient solution	Calcium in the nutrient solution	pH of expressed sap				
		Lettuce		Tomato		
		Shoots	Roots	Leaves	Stems	Roots
	p.p.m.					
pH 4	20	6.1	6.1	6.0	6.0	6.4
	80	6.1	6.1	6.1	6.1	6.6
	280	6.1	6.2	6.2	6.1	6.5
pH 5	20	6.1	6.1	6.1	6.1	6.6
	80	6.1	6.2	6.2	6.1	6.4
	280	6.1	6.1	6.1	6.0	6.3
pH 6	20	6.2	6.2	6.1	6.0	6.4
	80	6.1	6.0	6.1	6.1	6.3
	280	5.9	6.0	6.0	6.0	6.2

nutrient solution had no effect, within the limits of experimental error, on the pH of the sap.

Discussion

As pointed out previously, the main efforts in this work were directed toward securing data, which by distinguishing between the direct and indirect effects of hydrogen-ion concentration, would surmount the obvious difficulty in evaluating the true effects of the external reaction on plant growth. The data under discussion were obtained by means of an experimental technique that allowed for the variation of hydrogen ion concentration between pH 3 and 9, while the concentration of all nutrients (except sulphate) remained constant. Two principal conclusions can be drawn: First, that fatal or profoundly adverse effects of external reaction are encountered only at extremes of acidity and alkalinity (pH 3 and 9); and sec-

ond, that, with certain reservations, fluctuations in hydrogen ion concentration *per se*, within the relatively wide range of pH between 4 and 8, are not inimical to the welfare of the plants so far studied.

These effects of hydrogen ion concentration on growth are explicable by the results of the experiments on the relation of hydrogen ion concentration to nutrient absorption (**3**). It is not implied that the relation of external reaction to nutrient absorption is to be regarded as the sole or even the principal cause of the reported effects of pH on growth. Nor is it at all certain on the basis of the present evidence that the influence of pH on absorption is not in itself a reflection of a primary injury to the absorbing root cells. What is noteworthy, however, is the fact that the evidence of the absorption experiments can in itself account for the observed growth responses.

It was shown (**3**) that at pH 3 the plants were unable to absorb calcium and phosphate and at pH 9 phosphate absorption was drastically curtailed. At pH 4 and 5, but not at the less acid reactions, tomato and lettuce showed a diminished calcium absorption (**3**).

These findings can account for the failure of growth at pH 3 and 9 and for the favorable effect on growth of an increased calcium supply at acid reactions (fig. 4). They also suggest that at the extremes of the relatively wide, physiologically suitable range of external pH between 4 and 8, good growth is possible only if special safeguards assuring a favorable supply of nutrient ions are observed. On the acid side a high concentration of calcium in the nutrient medium is a prerequisite to good growth. Above pH 7 special attention must be given to the supply of the heavy metals (iron, manganese) which, unless made available from such sources as humate compounds, may become deficient.

Likewise, the decreased absorption of phosphate at alkaline reactions raises the question whether, similar to the case of calcium at acid reactions, a higher phosphate concentration at reactions between pH 7 and 9 would in turn lead to improved growth. We have no evidence on this point at present but it is to be noted that, unlike calcium at acid reactions, the limited solubility of phosphates at alkaline reactions precludes any material increases in concentration of soluble phosphate in the nutrient solution. Similar questions might be raised with regard to the supply of heavy metals at alkaline reactions, particularly at pH 8, at which the reduction in the absorption of phosphate is perhaps not great enough to account for the decline in growth. The absorption of Ca, Mg, K and NO_3 at pH 8 was comparable to that at more acid reactions.

The evidence under consideration lends support to the view that the effects of external reaction on growth are largely dependent on the other components of the nutrient medium [compare (**4**)]; by proper attention to them, an initially adverse response to a certain hydrogen ion concentration

can be favorably modified. There is, however, some justification for regarding the pH range above 5 and below 7 as particularly suitable for the growth of higher plants. This does not imply that good growth outside this range is impossible or even difficult to obtain. In fact, ample evidence to the contrary is available. Within this range, however, good growth is possible with least attention to questions of availability and suitable concentration of nutrients in the culture medium. Only in this restricted sense can we regard an external reaction around pH 6 as being optimal. These observations are in harmony with the results of an earlier investigation (**1**), which failed to disclose a definite optimal pH with either ammonium or nitrate nitrogen. It is recognized that, in the absence of special measures, the initial pH of the nutrient medium is subject to change as a result of the metabolic activities of the plant including nutrient absorption. This is of course relevant to all discussions of effects of the external reaction on plant growth.

Experimental verification was found for the point of view that the pH of a soil—ignoring for the moment the difficulty of measuring or deciding what the pH of a soil really is—can only have meaning when interpreted not as an independent variable but as a measurement reflecting the interaction of several factors peculiar to a given soil. It was shown that within relatively wide limits an acid or alkaline reaction in itself does not necessarily result in a serious impairment of growth. The pH of a soil is a helpful measurement in predicting and interpreting plant responses in so far as it serves as an indicator of the probable interrelations between plant nutrients. The significance of an acid pH in a soil will be determined by the extent to which it reflects a deficiency of calcium, and not infrequently magnesium, or the presence in the soil solution of toxic amounts of aluminum or manganese. On the other hand, the interpretation of an alkaline soil reaction cannot easily be differentiated from the question of availability of such plant nutrients as phosphate, iron or manganese.

If these premises are accepted, it follows that generalizations about plant responses to a given soil reaction are valid only for soils in which these indirect effects of reaction are similar. Little can be gained from comparing the reactions of soils which differ in parent material, climatic conditions prevalent during weathering, buffering capacity, organic matter content, etc. To cite only one example, good growth is encountered in certain acid peat or muck soils known for their high calcium-supplying power whereas a similar acid reaction is generally associated with crop failure in all leached, mineral soils. Indeed, the question may be legitimately raised whether the observed adaptations of the so-called acid-loving and alkali-tolerant plants to particular soil reactions should not be more appropriately related either to a high or a low requirement of some nutrients such as calcium, iron, and

manganese or to the relative tolerance of some toxic constituent such as aluminum. In this connection it will be of interest to institute further inquiries on the relation of organic matter to the availability of metals in the form of humates under alkaline soil conditions. The assigning of definite minimum, optimum, and maximum pH values to specific crops, based on experience with some particular soils, is open to question.

Special mention should be made of calcium nutrition. It was found that at acid reactions lettuce and tomato required for best growth a higher concentration of calcium than at reactions approaching neutrality. This observation is of particular interest since a high acidity in soils generally coincides with a low calcium supply. The plant thus suffers from a double disadvantage. Its increased physiological demand for calcium is met by decreased supply. It seems reasonable to associate in part at least, the good growth sometimes encountered in acid mucks or other acid soils rich in organic matter to the high calcium-supplying power of these soils. These considerations of some of the aspects of soil reaction may be appropriately concluded with a restatement of the difficulty in determining the true hydrogen ion concentration of the soil—root interphase.

The observed stability of the pH of expressed sap of plants grown in a wide range of external reactions is in accord with other experiments reported from this laboratory (**2, 6**).

Summary

A water culture method is described for the study of the effects of external hydrogen ion concentration within the range of pH 3 to 9. Its essential features provided, among others, for the maintenance of the same concentration of calcium, magnesium, potassium, nitrate, and phosphate ions as well as for the availability of iron and manganese throughout the entire pH range.

The results show that profoundly adverse effects of hydrogen, or hydroxyl, ion concentration, isolated from other variables, are found only at extremes of acidity or alkalinity. With tomato, lettuce, and Bermuda grass, complete failure of growth occurred at pH 3 and a marked decline was observed at pH 9.

Within a relatively wide range of pH between 4 and 8, fluctuations in the hydrogen ion concentration are tolerated by plants provided an adequate supply of all nutrient elements is maintained.

Growth of tomato and lettuce in acid nutrient solutions at pH 4 and 5 was favorably affected by increasing the concentration of calcium in the nutrient solution. At pH 6, however, the growth obtained at low and high concentrations of calcium was equally favorable.

The external reaction of the nutrient solution had no significant effect on

the pH of the expressed sap of either shoots or roots, with the possible exception of extremes unfavorable for growth.

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CARBOHYDRATE AND NITROGEN TRENDS IN BLUEBUNCH WHEATGRASS, *AGROPYRON SPICATUM*, WITH SPECIAL REFERENCE TO GRAZING INFLUENCES

SAMUEL K. MCILVANIE

(WITH EIGHT FIGURES)

Introduction

A knowledge of the growth requirements of a grass has been considered of fundamental value in adjustment of grassland management. Plant food reserves have been emphasized because the plant is most vulnerable at the depleted level of stored plant foods. A study of the trends of carbohydrates and nitrogen associated with growth stages provides basic material for determination of growth requirements. Such trends were studied in bluebunch wheatgrass.

Bluebunch wheatgrass, *Agropyron spicatum* (Pursh) Scribn. and Smith, composes 85 per cent. of the total vegetation in climax grasslands of southeastern Washington (**6**); further, it is outstanding in the sagebrush, spring-fall ranges of central Washington and Idaho. It is an important forage plant found on dry slopes, and in canyons, open woods, and sagebrush lands from northern Michigan to Alaska, south to western South Dakota, New Mexico, and California (**14**). Unrestricted grazing has resulted in serious deterioration of millions of acres of former wheatgrass-sagebrush ranges (**9**).

The following aspects of growth were considered and are herein reported: (1) Observations on external growth characters and mechanisms; (2) seasonal trends of carbohydrates and nitrogen in roots and herbage of ungrazed plants; (3) the effects of frequent spring clipping on carbohydrate and nitrogen content.

Carbohydrates are usually considered the most important reserves; several questions exist, however, with reference to specific fractions as reserves. Hemicelluloses and reducing sugars were considered important reserves by ENGARD (**7**) in studies with annual canes of raspberries, whereas starch and sucrose were not. Sucrose was considered important in metabolism by SAMPSON and MCCARTY (**21**) in the herbage of *Stipa pulcra*. MCCARTY (**17**) considered sugars and "starches" to be the most important reserves in *Bromus carinatus*, while hemicelluloses were not so considered. HANSON and STODDART (**11**) found little difference in the hemicellulose content of grazed and ungrazed plants of beardless wheatgrass although marked differences were noted in sugars and "starch."

The percentage of carbohydrates in mountain brome (**17**) reached a maximum in roots and herbage immediately following the current seasonal growth, whereas minimum carbohydrate content occurred during the initial

stages of shoot formation. Early growth was made wholly at the expense of stored carbohydrates, which did not reach their maximum values until after growth rates declined. SMELOV (**22**) found carbohydrates to increase in herbage of grasses up to flowering, to hold through to seed ripening, and to decline during dying off.

The effects of clipping and grazing have been noted by many investigators. PARKER and SAMPSON (**19**) found that yield was decreased most by frequent clipping during the period of maximum growth rate. Root development was directly affected by the amount of leaf surface. ALDOUS (**1**) reported that protection of *Andropogon scoparius* until August and then clipping every two weeks, and clipping every two weeks until August and then protection, produced equal degrees of depletion. GRABER (**10**) found optimum reserves were associated with increased competitive ability in bluegrass.

Less emphasis has been placed on nitrogen as a reserve. LONG and MURNEEK (**15**) concluded that the roots of strawberry plants store 30 to 40 per cent. of the total nitrogen used in spring growth. NIGHTINGALE (**18**) reported a decrease of amino acids and an increase in amides under conditions of carbohydrate deficiency. He quotes PRIANISCHNIKOV for the statement that formation of ammonia and amides in plants is comparable to the formation of urea in animals.

A review of the literature indicates that reducing sugars, sucrose, and reserve polysaccharides (mainly hexosans) may be considered most important in a reserve food study of grasses. These fractions plus nitrogen determinations were used in an effort to determine the most vulnerable stage of bluebunch wheatgrass. Vulnerability is used to describe that stage in which the plant is presumably least able to survive removal of photosynthetic area.

Experimental area

The experimental area was located at the Bridger Ranger station of the Gallatin National Forest, Bozeman, Montana. The vegetation was a climax bunchgrass type of local extent, representative of the transition from the sagebrush-bunchgrass community to an open woodland of xeric timber species such as limber pine, *Pinus flexilis*. Individual clumps of bluebunch wheatgrass were scattered at intervals of about three feet in an irregular pattern. A mixture of species occurred in the interspaces including arrowleaf balsamroot, *Balsamorhiza sagittata*, and Idaho fescue, *Festuca idahoensis*. Other important species were *Poa secunda*, *Stipa columbiana*, and *Artemisia tridentata*.

Monthly precipitation and temperature data for the study period are presented in table I. Mean monthly temperatures and monthly precipitation are shown graphically in the form of a hythergraph (**5**, fig. 1). March was

TABLE I

MONTHLY PRECIPITATION AND TEMPERATURE DATA FOR THE EXPERIMENTAL AREA, MARCH TO NOVEMBER, 1939

MONTH	TOTAL PRECIPITATION	MEAN MAXIMUM TEMPERATURE	MEAN MINIMUM TEMPERATURE	MEAN MONTHLY AVERAGE TEMPERATURE
	in.	° *F.*	° *F.*	° *F.*
March	1.02	43.3	21.9	32.6
April	0.99	59.1	32.5	45.8
May	1.91	67.7	40.6	54.1
June	3.71	65.8	42.2	54.0
July	0.69	81.0	51.2	66.1
August	0.94	80.5	48.5	64.5
September	1.29	70.6	41.5	56.2
October	1.17	57.3	32.7	45.0
November	0.05	52.5	25.4	38.9

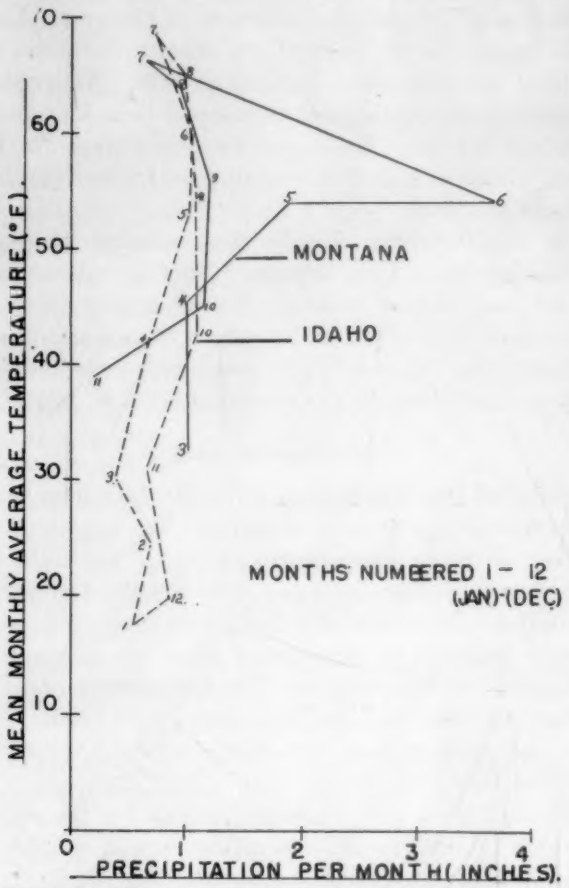

FIG. 1. Hythergraph of Bozeman, Montana (March to November, 1939) and southern Idaho (average for period 1924–34).

the month of lowest temperatures for the period. April and March received approximately one inch of precipitation each, whereas May and June were the wettest months with 3.7 inches for the latter. July, August, and early September included periods of high temperatures.

Climatic conditions for the study area are compared with those of a southern Idaho bluebunch wheatgrass community in figure 1. It is of interest to note that these widely different areas have a similar midsummer drought, an important feature of the climate for bluebunch wheatgrass. Higher total precipitation allows for a more mesic complement of species in the Montana study area.

Methods

Samples were collected at two-week intervals from plants growing *in situ* on an area which had been protected from grazing for many years. Prior to initiation of sampling, all plants were clipped to crown level to remove previous top growth. A sample consisted of one bunch (roots and herbage) for each two-week interval; each bunch contained 75–100 individual plant units held together by a matrix of dead plant material. Roots were taken to a depth of 1 to 1.5 feet.

Unclipped plants were compared to plants clipped at two-week intervals, at crown level, for an eight-week period beginning with the 2.5-inch height stage.

Measurements of crown diameter and culmless height growth were made on each plant taken. Phenological development was recorded. "Herbage" includes all tissue more than one-half inch above the root collar; "roots" includes the remaining stem base and roots proper. As no aerial green growth was present on the first clump of plants taken, a separate fractioning was made by dividing the aerial portion into lower internodes, and the remaining tops. The plants were taken to the laboratory at once, separated into the individual plant units, washed free of dirt with cold water, and then divided into the fractions.

The root samples varied from 18 to 40 grams of oven dry material and the herbage from 7 to 30 grams. The fractions were dried *in vacuo* at 70 degrees C., ground to pass through a 1-mm. mesh, and stored in stoppered bottles.

Duplicate 2-gram samples were taken of each plant fraction for the determination of reducing sugars, sucrose, and reserve polysaccharides. Samples were extracted over 75 per cent. alcohol in a Soxhlet extractor for two hours, the extract removed, more alcohol added, and extraction continued for four hours. The extracts were used for sugar determinations, and the residue for reserve polysaccharides. The ceric sulphate method as outlined by BARR (**2**) and HASSID (**12**) was followed for determination of reducing sugars and sucrose. A commercial invertase solution was used for inversion

of sucrose. The difference between total sugars in the sucrose titration and the reducing sugars is reported as sucrose. The residue from the alcohol extraction was mixed with water, heated on a water bath, cooled, and treated with diastase; after clearing, the filtrate was hydrolyzed with 10 ml. of concentrated hydrochloric acid to 200 ml. of solution. The results are reported as reserve polysaccharides and may include principally dextrins and levulins.

The standard Kjeldahl method modified to include nitrates and nitrites was used on 2-gram samples of ground plant materials (**16**). CULLEN-VAN SLYKE tubes were used in determination of amide nitrogen (**16**) on a limited number of key samples.

All data, except the graph showing the trend of total nitrogen, and the clipping results, are reported on the basis of percentage of oven dry weight.

TABLE II

DRY MATTER CONTENT OF ROOTS AND HERBAGE OF BLUEBUNCH WHEATGRASS FROM MARCH TO OCTOBER, 1939

GROWTH STAGE	DATE COLLECTED	PLANT	PERCENTAGE DRY MATTER	
			TOPS	ROOTS
			%	%
1	Mar. 25	1	28.2	31.9
2	Apr. 4	2	22.9	29.5
	" 14	3	24.0	29.8
	" 25	4	18.5	25.2
	May 5	5	16.0	25.5
	" 15	6	22.0	19.4
	" 29	7	29.0	31.0
3	June 10	8	25.7	19.0
	" 20	9	26.8	20.7
	July 3	10	28.7	27.3
	" 11	11	33.0	22.9
	" 25	12	38.5	27.3
4	Aug. 8	13	23.0	21.3
	" 21	14	27.0	21.5
	Sept. 5	15	36.0	26.3
	" 18	16	33.8	25.2
5	Oct. 4	17	24.5	25.5
	" 18	18*	.....	.....
	Nov. 1	19*	.....	.....
	" 13	20*	.....	.....

* Data not available.

Sample graphs of carbohydrate trends using green and dry weight bases, presented the same general picture. The data were originally prepared using the dry weight method, therefore, only those results which were altered by green weight interpretation have been changed to the latter. Table II presents the percentage of dry matter in the respective clumps of plants.

Experimental data and results

The following growth stages are used throughout the paper:

1. Growth initiation or formative stage. No external green growth, although pale shoots about 0.5 inch long were hidden in the crown, snow disappearing; ground frozen except for the surface inch. The rate of increment shown in figure 2 indicates that only 3 per cent. of the total height growth was present at this stage.

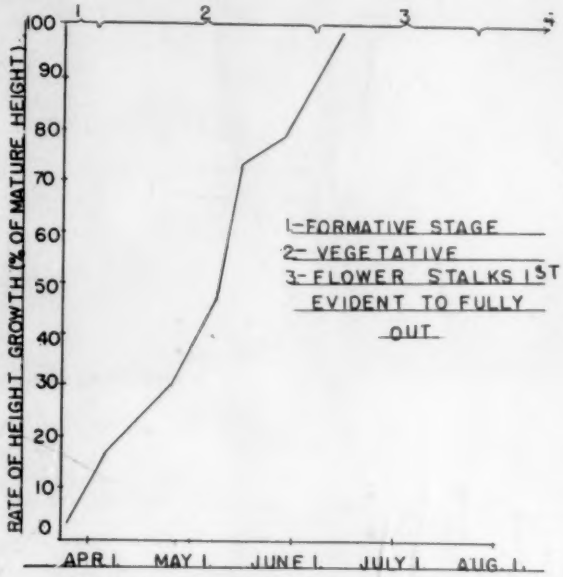

FIG. 2. Increment rate; mature height = 100.

2. Vegetative development. About two months were required to complete the vegetative development; essentially all the height growth was reached in this period.

3. Flower stalks first evident to fully out. Culmless herbage increment ceased, and flowering and seed formation occurred.

4. Seed maturation and early yellowing. Descriptive of the brief rest period prior to secondary growth.

5. Secondary growth (aftermath). Production of green shoots from basal buds which is characteristic of bluebunch wheatgrass. Variable climatic conditions greatly increase or decrease this stage in different years. Considerable secondary growth occurred in the fall of this study.

Extension of the bunch, in part, is vegetative reproduction as shown in figure 3. Prior to emergence of basal shoots, shoots developed from the second and third nodes of the previous season growth. Unlike the brittle stems above, the internodes below these shoots were yellow, dry, waxy, and

firm. By May 5, the shoots were 7 inches high and producing adventitious roots which established new plant units. The magnitude of such vegetative activity is partially indicated in the spread of crown diameters, which ranged from 3 to 9 inches. Over half of these were in the 4- to 5-inch diameter class, which represents the ordinary climax bunch for the study area. The amount of dead central core tended to increase with the diameter.

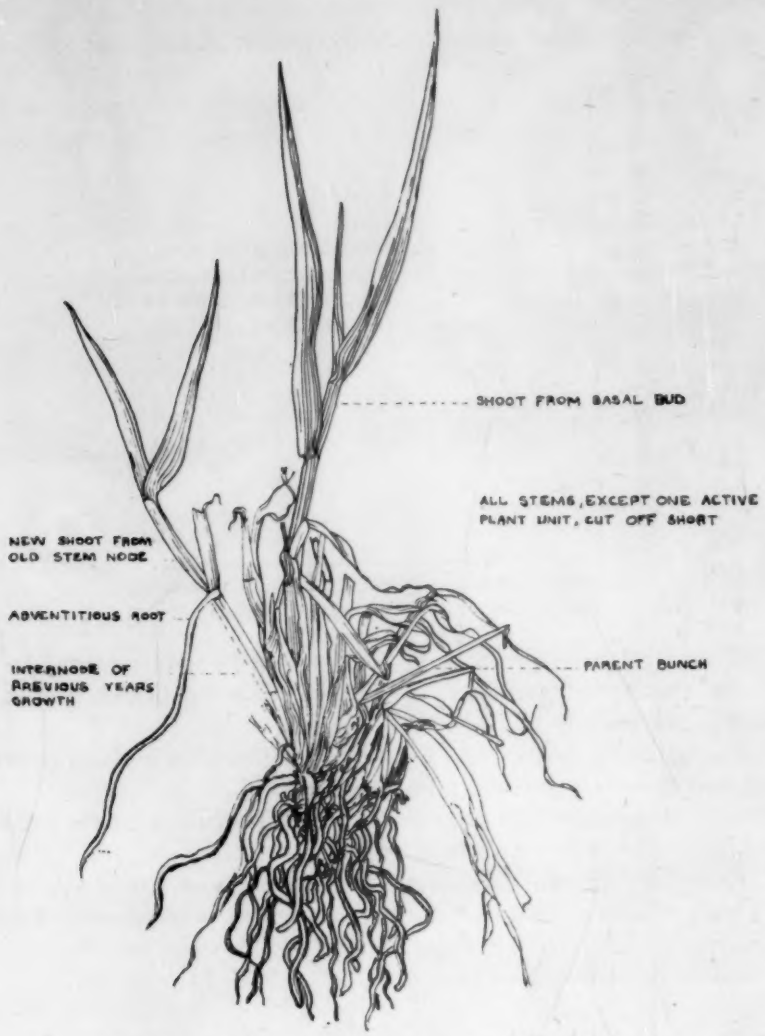
SHOOT FROM BASAL BUD
ALL STEMS, EXCEPT ONE ACTIVE PLANT UNIT, CUT OFF SHORT
NEW SHOOT FROM OLD STEM NODE
ADVENTITIOUS ROOT
INTERNODE OF PREVIOUS YEARS GROWTH
PARENT BUNCH

FIG. 3. Vegetative reproduction in *Agropyron spicatum* showing a new plant unit formed from the node of the previous year's growth.

Carbohydrate trend

Figures 4 and 5 present the seasonal trends of the carbohydrates with reference to growth stages. For convenience, the trend is outlined by successive growth stages.

Formative stage.—Maximum seasonal carbohydrate content of 3.15 per cent. was present in the roots. The lower two to three internodes contained 3.25 per cent. total carbohydrates, whereas the balance of the old herbage contained 1.27 per cent. Sucrose was present in quantities greater than reducing sugars or reserve polysaccharides.

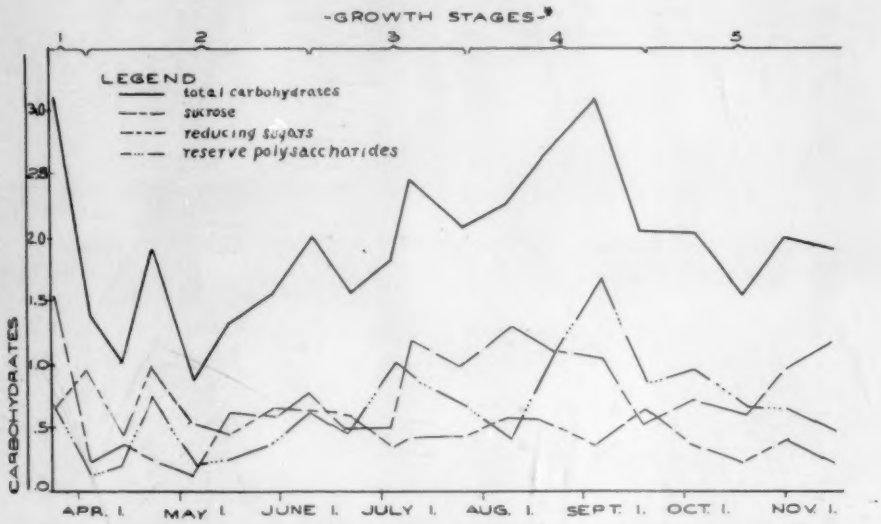

Fig. 4. The trend of carbohydrate in roots of bluebunch wheatgrass; percentage dry weight. (1) Initiation of shoots, ground frozen, snow disappearing; (2) Vegetative development; (3) Flower stalks evident to fully out; (4) Seed maturation, early yellowing; (5) Secondary growth.

Vegetative developments.—Sucrose and reserve polysaccharides declined sharply to a minimal position during most of this stage. Reducing sugars maintained generally a high level in herbage and roots. The seasonal minimum content of total carbohydrates was reached during this period.

Flower stalks first evident to heads fully out.—The seasonal culmination of total carbohydrate content of 4.68 per cent. occurred in the herbage; at the same time, the total carbohydrate content in the roots was 60 per cent. of the final maximum. Reducing sugars were in maximum relative position at the onset; after a marked decline, however, sucrose became of most importance.

SEED MATURATION TO EARLY YELLOWING.—Rapid decline in herbage total carbohydrates occurred during this stage. The roots, however, reached their seasonal maximum of 3.11 per cent. total carbohydrates. The brief rest period was evidenced by an increase in reserve polysaccharides, and decrease in sucrose in roots and herbage.

FALL CURING AND SECONDARY GROWTH.—Initial secondary growth coin-

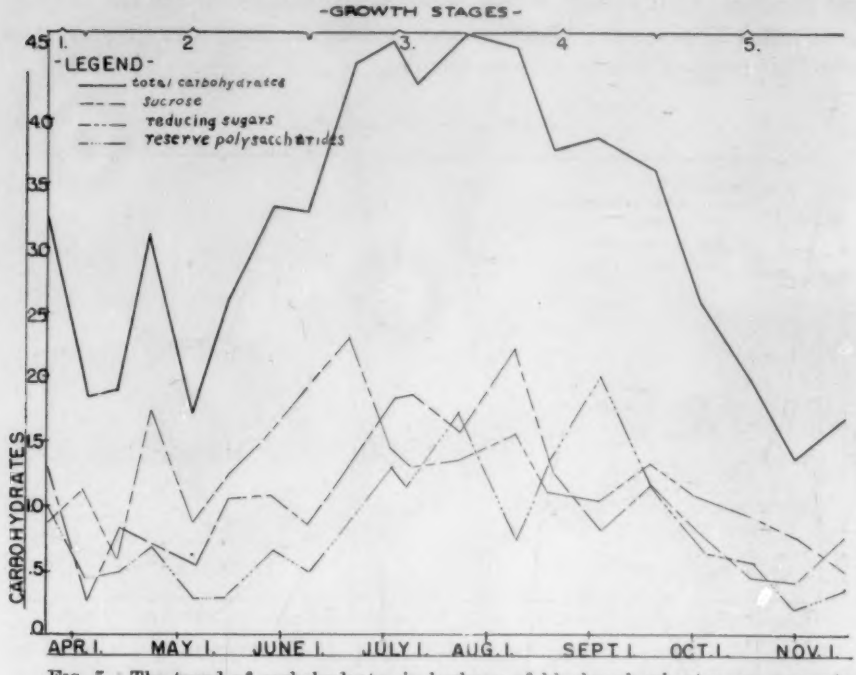

FIG. 5. The trend of carbohydrates in herbage of bluebunch wheatgrass; percentage dry weight.

cided with a sharp decline in reserve polysaccharides in roots and herbage. The relative amounts of the carbohydrate fractions closely approached the condition noted above in vegetative development. A slight increase in total carbohydrates occurred in herbage and roots toward the close of this stage.

TOTAL NITROGEN TREND

In figure 6 the results of total nitrogen determination for herbage and roots are reported. Only those plants which represented significant points on the carbohydrate seasonal curve were analyzed. The nitrogen trend is reported by growth stages using the green-weight curve (fig. 6).

FORMATIVE STAGE.—Total nitrogen in roots and herbage were approximately equal and near seasonal maxima.

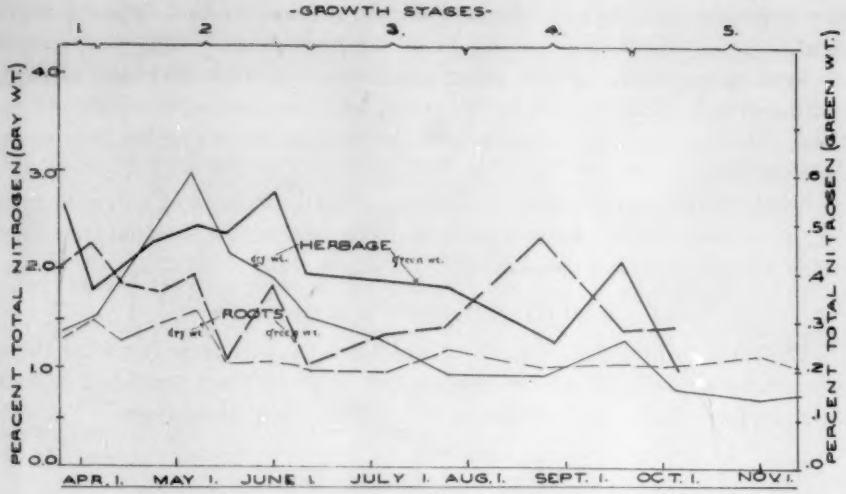

FIG. 6. The trend of total nitrogen in bluebunch wheatgrass.

VEGETATIVE DEVELOPMENT.—Herbage nitrogen maintained a steady rise, after a sharp drop in the formative stage, to a peak at the close of the

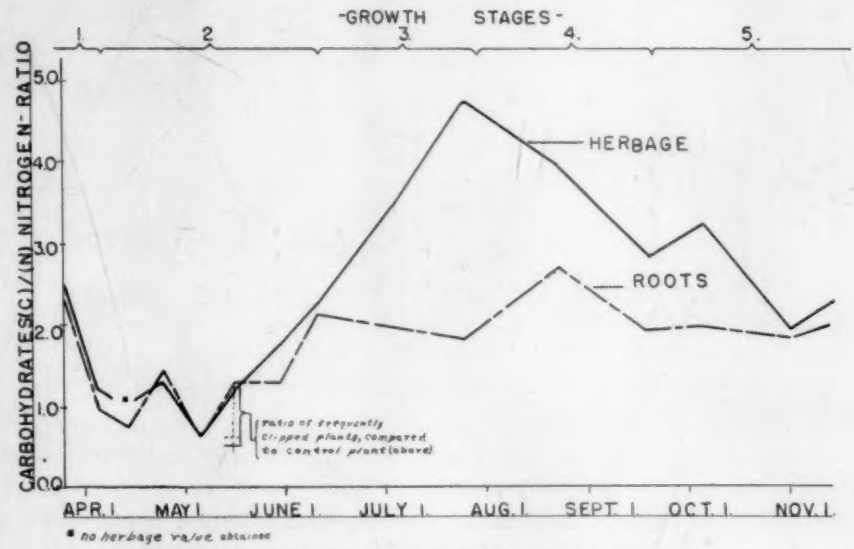

FIG. 7. Trend of the C/N ratio in bluebunch wheatgrass.

vegetative stage; root nitrogen, however, maintained an opposite downward trend to a low point at the close of the vegetative stage.

FLOWER STALKS FIRST EVIDENT TO HEADS FULLY OUT.—Herbage nitrogen

slowly declined, while root nitrogen showed a more marked opposite movement.

SEED MATURATION TO THE EARLY YELLOWING PERIOD.—Herbage nitrogen continued to decline to a low in this stage, while root nitrogen continued upward. At the close, the situation was reversed in the transition into secondary growth.

FALL CURING AND SECONDARY GROWTH.—Small amounts of nitrogen present; roots made slight increase, while herbage reached the seasonal minimum. Green weights were not available for this stage.

CARBOHYDRATE-NITROGEN RATIO TREND

The total carbohydrates (sum of three fractions reported) ÷ total nitrogen is termed the C/N ratio of this study. Figure 7 is a graphical presentation of the trend in herbage and roots of bluebunch wheatgrass.

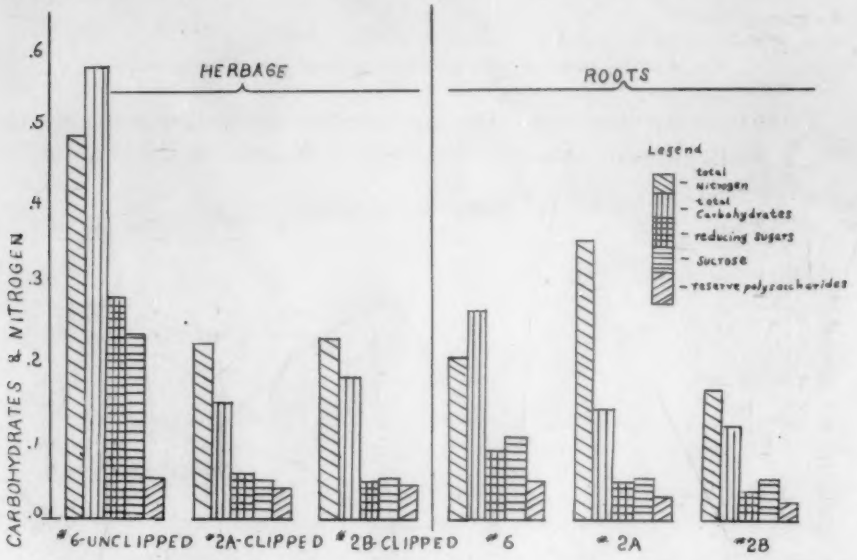

FIG. 8. Carbohydrates and nitrogen in clipped and unclipped plants; percentage of green weight.

Initially, the ratio was high, 2.45 and 2.39, in herbage and roots, respectively. During the greatest vegetative activity, lowest ratios occurred (0.67 and 0.56) for herbage and roots. During differentiation a maximum of 4.8 occurred in the herbage. HICKS (**13**) has observed a similar relationship in wheat plants. At the close of the study the ratios were approximately equal to those of the first plant in the formative stage.

CLIPPING STUDY

The clipped and control plants were compared for carbohydrates, total

nitrogen, and amide nitrogen. Tables III and IV, and figure 8 present the results of the clipping experiments using green-weight basis.

The total carbohydrate content in roots of the unclipped control was

TABLE III

COMPARISON OF CLIPPED AND UNCLIPPED PLANTS OF BLUEBUNCH WHEATGRASS. ALL PERCENTAGES BASED ON FRESH WEIGHT

PLANT NUMBER	C/N RATIO	TOTAL NITROGEN	CARBOHYDRATES			
			REDUCING SUGARS	SUCROSE	RESERVE POLYSACCHARIDES	TOTAL
ROOTS						
		%	%	%	%	%
6 (unclipped)	1.27	0.204	0.091	0.119	0.049	0.259
2A (clipped)	0.38	0.357	0.049	0.051	0.035	0.135
2B "	0.77	0.163	0.041	0.054	0.030	0.125
HERBAGE						
6 (unclipped)	1.18	0.480	0.278	0.227	0.063	0.568
2A (clipped)	0.52*	0.275*	0.051	0.046	0.045	0.142
2B "	.....	.....	0.047	0.048	0.044	0.139
2A "	.....	0.570†	.....	.....	.....	.....
2B "	.....	0.412	.....	.....	.....	.....

* Average for 2B and 2A.
† A composite of all clippings.

TABLE IV

COMPARISON OF AMIDE NITROGEN IN ROOTS OF CLIPPED AND NON-CLIPPED PLANTS OF BLUEBUNCH WHEATGRASS*

PLANT	DATE COLLECTED	TOTAL CARBOHYDRATE	AMIDE NITROGEN	REMARKS
		%	%	
1a	Mar. 25	.....	0.0011	First plant taken, no clipping, no green growth evident
1b	"	1.04	0.0019	
6a	May 15	.....	0.0058	11 inches total height growth, no clipping, normal growth
6b	"	0.259	0.0055	
2Aa	May 15	.....	0.0046	Clipped on April 7, 14, 25, May 5 with regrowth removed each clipping
2Ab	"	0.130	0.0039	

* All percentages based on green weight.

0.259 per cent. compared to an average of 0.130 in the clipped plants; a 50 per cent. reduction. The herbage showed a reduction of 77 per cent. carbohydrates in the frequently clipped plants.

The clipped plants had 43 per cent. less total nitrogen in the herbage than did the unclipped control; conversely, the clipped plants had 22 per cent. more total nitrogen in the roots than did the control plant; the latter figures were erratic, however. The composite of all clippings from the clipped plants was 0.49 per cent. nitrogen.

No striking difference occurred in amide nitrogen. The clipped plants averaged 0.0042 per cent., and the control 0.0056 per cent. amide nitrogen. The plant in the formative stage had 0.0015 per cent.

Frequent clippings influenced the C/N ratio as noted in table III. The control plant had ratios of 1.27 and 1.18 for roots and herbage, whereas the clipped plants averaged 0.57 and 0.52. The sum of heights of all clippings was 12 inches; the height of the control plant was 11 inches.

Discussion

Basal stem storage and vegetative reproduction

Translocation of carbohydrates in the late fall removed most of that fraction from the upper herbage, while the lower two or three internodes contained quantities slightly greater, on a percentage basis, than that found in the roots. The basal internodal reserve was used in vegetative expansion as shown in figure 3. Simple tillering, however, appears to account for the greater part of bunch expansion; a range of 3 to 9 inches in bunch diameter occurred. Variations in fall growing condition may alter storage in basal stems since bluebunch wheatgrass often grows throughout much of the open winters. As will be noted later, the last plants studied did not have as much stored reserves in the stems as did the first.

Trends of carbohydrates and nitrogen

One of the two maxima of carbohydrate content was found in the roots of the plant first studied (formative stage). Sucrose was most abundant at this time. The work of McCarty showed the importance of sucrose and "starch" as storage forms in mountain brome (**17**). Total nitrogen was present in relatively high seasonal amounts and may be considered an important reserve.

Rapid decrease in total plant carbohydrates, and increase in herbage nitrogen occurred in the vegetative phase. Sucrose and reserve polysaccharides became relatively less abundant than reducing sugars, which indicates the importance of the former in storage. A close parallel in changes of carbohydrates in roots and herbage indicated that internodal storage may

be used in hydrolysis and translocation since the herbage at this stage consisted largely of the internodes of the previous year's growth.

The most vulnerable period of seasonal development was reached during the vegetative stage. Two carbohydrate lows were reached in the roots at the 4- and 7-inch height stages, which represented depletions to 29 and 27 per cent., respectively, of the total reserves. Root-nitrogen content followed a similar trend although the herbage showed a steady rapid increase (fig. 6) culminating at the close of the vegetative stage. It is of interest to note that in mountain brome a 25 per cent. residual of total carbohydrates was also present at the seasonal minimum (**17**). The minimum C/N ratio of 0.56 and 0.57 for roots and herbage was present at the 7-inch stage. The point at which carbohydrate content of roots and C/N ratio were minimum was considered the most vulnerable point in the seasonal development; namely, the 7-inch stage. Since height variation occurs from year to year, the vulnerable point may be considered as occurring in the middle of the vegetative stage. The dry matter content of the herbage was also lowest at this point. In a study of the nutritional value of the 3- to 5-inch, 7- to 10-inch, and headed stage in *Agropyron inerme* (very closely related to *A. spicatum*), BURKITT (**3**) found the former to produce faster gains on sheep. It will be observed that the 3- to 5-inch stage would be within the period of minimum carbohydrate reserves. The 7- to 10-inch stage would provide nutritious forage and still be past the vulnerable stage of 7 inches, or the middle of the vegetative stage. The peculiarities of grazing are such that grazing could begin at the most vulnerable point because of the lag in actual cropping of each plant, *provided* proper stocking exists. Incidentally, the work of animal nutritionists could be markedly improved if plant and animal relationships were analyzed on physiological unit bases, rather than present empirical units.

Approximately 45 per cent. of the current height growth was produced before root reserves ceased to decline, or until the most vulnerable point was reached. Only 10 per cent. of the current height growth in mountain brome grass (**17**) was at the expense of reserves. A greater dependence on reserves apparently exists in bluebunch wheatgrass as compared to mountain brome.

A period of catabolism has been described; hereafter, the anabolic phase is marked by increasing amounts of carbohydrates. Maximum seasonal carbohydrate content occurred in the third growth stage when root reserves were about 60 per cent. of the peak. The herbage high in carbohydrates coincided with the summer drought.

A study of graphs (figs. 4, 5) reveals three major relationships among the carbohydrate fractions. Reducing sugars were relatively most abundant during vegetative growth, while sucrose was dominant during seed

formation and maturation; later reserve polysaccharides were in peak position prior to secondary growth.

The appearance of the flower stalks marked a stage of lowered herbage nitrogen which had increased steadily during vegetative growth, while root nitrogen had moved in an opposite direction. The reversed situation in stages 3 and 4 is significant, in that it may indicate root development during the summer drought. The author has not checked this with field studies. Total root nitrogen, in the transition between stages 2 and 3, was depleted approximately 53 per cent. of the amount present in the formative stage; total root carbohydrates were depleted approximately 70 per cent. Nitrogen as well as carbohydrates appears to have been an important factor in the reserves. Similar observations are reported for the strawberry plant, (**15**), where the roots stored 30 to 40 per cent. of the reserve.

It is of interest that the two months lag between peak reserves for herbage and roots was not found in the case of mountain brome grass (**17**), where the maxima occurred simultaneously. The prominent summer drought of the bluebunch wheatgrass climate may account for this difference.

The brief rest period, in which reserve polysaccharides were most abundant, was ended by the period of secondary growth. The trends in reserves and activity of the carbohydrate fractions were essentially the same for secondary as for the initial vegetative growth. The magnitude of secondary growth varies according to the fall and winter climatic conditions. In certain years, green growth is present on bluebunch wheatgrass during the winter. Final reserves were not present at the close of the study, when 4 inches of active green material was included in the bunch. The roots did contain about 60 per cent. of the reserves which were present in the formative stage. Some depletion of final reserves by secondary growth is expected, and has been noted by McCarty (**17**). The depletion recorded for bluebunch wheatgrass is, however, somewhat greater than that attributed to the 4 inches of regrowth. The cause for this discrepancy is not apparent. Unfortunately, the study was not carried through the winter, although the author is of the opinion that such studies should be carried on even under snow conditions.

Clipping study

Under undisturbed growth a residual reserve of 21 per cent. of the maximum was present in the roots at the minimum level; whereas the frequently clipped plants averaged 12.5 per cent. residual carbohydrates (green weight basis). At the date the frequently clipped plants were taken, however, their root and herbage reserves were 50 and 72 per cent., respectively, below the control which was two weeks after the normal seasonal minimum. The frequent clipping therefore greatly reduced the root re-

serves and herbage reserves (lower internodes). The lethal point of reserve depletion was not determined. The clipping definitely prolonged and intensified the vulnerable point. Prolongation of this condition in a plant native to a climate of normal summer drought is of concern, since the plant could not rebuild the reserves in that particular year. The limited precipitation during the summer drought is generally not available for growth (**20**). Therefore, the full 7-inch height stage should be reached prior to grazing if the plants are to be used at the same period each spring. Earlier use could be made by providing a system of rotation-spring grazing as described by CRADDOCK and FORSLING (**4**).

The C/N ratio in herbage of the control plant was 1.18 compared to 0.63 for the clipped plants; whereas, the roots of the former had a ratio of 1.27 compared to 0.57 in the latter. Low ratios are characteristic of strongly vegetative growth generally (**8**) which apparently applies to the roots as well as to the herbage. How long health could be sustained on the vulnerable point was not determined.

Summary

Samples of bluebunch wheatgrass, *Agropyrum spicatum* (Pursh) Scribn. and Smith, were collected from March 25 to November 13, 1939, near Bozeman, Montana. Plants were collected at two-week intervals from snow disappearance until late fall in a fully developed, protected bunchgrass area. The following analytical data were obtained:

1. Storage of available carbohydrate reserves occurs in the lower internodes in percentage amounts nearly equal to root reserves.

2. Peripheral expansion of the bunch is partially a result of shoots and adventitious roots being produced from the lower nodes which establish a new plant.

3. The 7-inch height stage coincided with the minimum of carbohydrates in the roots, and with minimum C/N ratio in the plant; this point was considered most vulnerable in the seasonal development of bluebunch wheatgrass.

4. The appearance of the flower stalks marked the beginning of highest total carbohydrate content in the herbage, which was sustained about one month and then declined rapidly during fall curing and secondary growth. A two-months lag in maximum carbohydrate stores occurred in the herbage and roots, with the latter occurring last.

5. Total nitrogen content in the roots was depleted in the vegetative stage to 53 per cent. of that present in the formative stage; a 70 per cent. depletion of carbohydrates occurred at the normal minimum.

6. Greatest relative amounts of reducing sugars were associated with

rapid vegetative growth, of sucrose with differentiation, and of reserve polysaccharides with the brief rest period prior to secondary growth.

7. The maximum total herbage nitrogen content occurred at the close of the vegetative stage and declined to about 70 per cent. (fresh weight basis) of this value by the time of the appearance of the first flower stalks.

8. Clipping to crown level at two-week intervals during 8 weeks of the vegetative stage produced the following changes in the clipped as compared to the unclipped plants:

a. 50 per cent. less total carbohydrates in the roots;
b. 72 per cent. less total carbohydrates in the herbage;
c. 22 per cent. higher total nitrogen in the roots;
d. 53 per cent. less total nitrogen in the herbage;
e. C/N ratio of 1.27 (roots) and 1.18 (herbage) in control;
f. C/N ratio of 0.57 (roots) and 0.63 (herbage) in clipped plants;
g. No marked difference in amide nitrogen.

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THE PROXIMATE CHEMICAL CONSTITUENTS OF CITRUS WOODS, WITH SPECIAL REFERENCE TO LIGNIN[1]

F. M. TURRELL AND P. L. FISHER

(WITH THREE FIGURES)

Introduction

Valencia orange fruit, which matures during the summer in California, is subject to a disorder recognizable especially when the fruit remains on the trees for some time after maturation. In its early stages, this disorder is characterized by a reduction in the amount of available juice in the vesicles in the proximal end of the fruit due to the gelation of the sap. Later there is an increase in the thickness of the walls of the cells composing the interior of the juice vesicle (fig. 1). This change is accompanied by gas formation and by changes in cell wall constitution. When treated with phloroglucinol and hydrochloric acid, the cell walls give a brilliant red lignin reaction (**16**) not shown by normal cell walls. This disorder is called "granulation" (**1**).

Sixteen constitutents known to be present in the orange fruit were tested with phloroglucinol and HCl. Two of these constitutents, crude orange oil and citral, formed transitory cerise colors. In the juice vesicle the cerise color remained several hours. E. C. CROCKER has shown that yellow to red colors develop with certain compounds containing aldehydes. We have confirmed his tests and concur with the view that coniferal aldehyde or hadromal probably are responsible for phloroglucinol-HCl color reaction in wood. Although the cell walls of granulated juice vesicles gave a positive phloroglucinol-HCl reaction for lignin, the reaction might be due to some other compound. Isolation of lignin from juice vesicles is in progress.

In this connection it was of interest to know whether there might be a tendency in various citrus species, varieties, and rootstock combinations toward greater lignification in the tree itself. It has long been recognized by DR. H. J. WEBBER and others that fruit is affected by the type of rootstock upon which the scions are budded or grafted and that certain rootstock and scion combinations are incompatible. Thus far-reaching changes might be expected to take place in the scions. Since no data on the constituents of citrus woods were available, this investigation was undertaken.

Materials and methods

Bole-wood samples (two from each tree) were collected from single trees of healthy Valencia orange, Washington Navel orange, and Marsh grape-

[1] Paper no. 460, University of California Citrus Experiment Station, Riverside, California.

fruit on sweet- and on sour-orange rootstock, and from Eureka lemon on sweet-orange and on Rough-lemon rootstock. Samples were also obtained from four psorosis(virus)-infected Valencia orange trees on sour-orange rootstock. Two of these trees showed leaf symptoms of the disease only, but through budding experiments they were known to contain the virus; the other two trees showed severe bark symptoms.

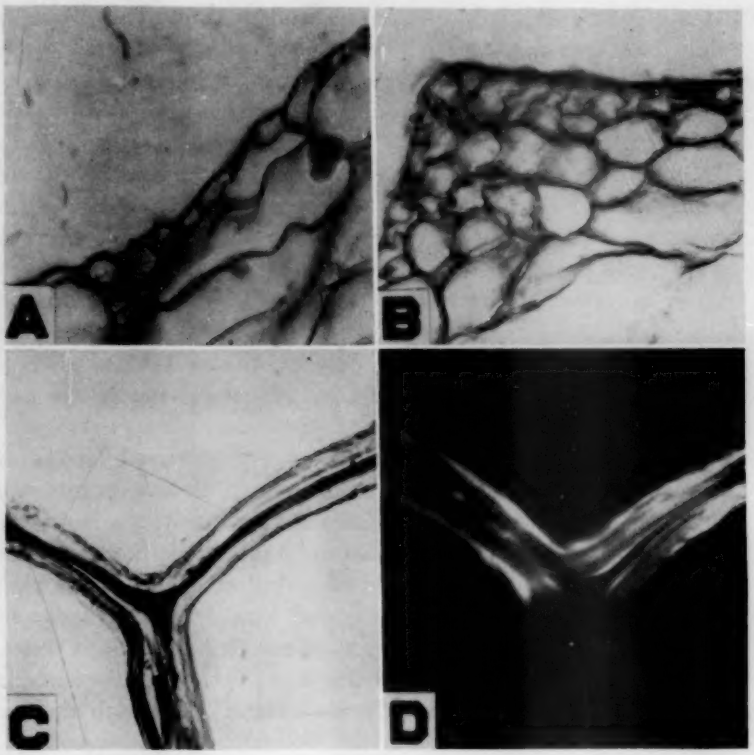

FIG. 1. Cell walls of normal and granulated Valencia orange juice vesicles. A. Thin cell walls along the edge of a normal juice vesicle (×336). B. Thin cell walls at the corner of a normal juice vesicle (×336). C. Thick cell walls found in the center of a granulated juice vesicle (×336). D. Same cell walls from the center of a granulated juice vesicle, photographed under polarized light. The double refractive cellulose is bright, while the lignified portions of the wall are nonrefractive and dark.

The samples, consisting of pieces $\frac{3}{4}$ inch × $\frac{3}{4}$ inch × 5 inches in size, were cut vertically from the sapwood of the scion portion of the tree trunk about $1\frac{1}{2}$ feet above the ground. Twigs were selected from two healthy Valencia orange trees on sour-orange rootstock, where the trunk wood was not sampled.

All trees were located in the experimental plots at the Citrus Experiment Station and were approximately twelve years old, except the psorosis(virus)-infected Valencias, which may have been somewhat younger.

The bark, which was shown by tests with $FeCl_3$ on water extracts to contain tannins, was stripped off all samples except the twigs before the wood was splintered and dried. The air-dried wood was then ground in a Wiley mill until fine enough to pass through an 80-mesh sieve and was dried at 105° C. for 48 hours in thin layers in shallow aluminum drying dishes. Forty-eight hours was determined to be the shortest drying time required to obtain practically constant weight. Longer drying periods gave satisfactory results up to that of 120 hours, when there were additional slight losses of weight.

In drying woods other than citrus, PARR and DAVIDSON (**10**) found that volatile constituents other than water accounted for a portion of the loss in dry weight. In the light of their findings, it is probable that volatile oils are also lost in drying citrus woods and must be included in the moisture lost. Since, as recognized by PARR and DAVIDSON, dry weight is important in certain calculations, great care must be exercised in obtaining a satisfactory working value. While every precaution was used, much is yet to be desired in this determination, especially with reference to the ash constituents.

The proximate inorganic constituents were determined on the ash of thoroughly oven-dried ground wood. Samples of 2 to 3 grams of wood were weighed into tared porcelain crucibles and ignited over a Bunsen flame. When the ash began to whiten (after 20 to 30 minutes), the crucibles were transferred to a muffle furnace heated to a dull red, and combustion was continued 4½ hours. They were then cooled in a desiccator and weighed. The percentage of ash was calculated on the basis of the weight of the sample of oven-dried wood. Care was used in keeping the samples the same length of time in the drying ovens and in the desiccator. Although the samples were in tightly closed aluminum drying dishes, the woods were so highly hygroscopic that they gained weight in the desiccator and during the weighing.

Spectrograms were made by volatilizing the ash of the various woods in a carbon arc and passing the light produced through a diffraction-grating spectrograph equipped with a rotating shutter. In order to make the intensities of the lines of the elements comparable between pairs of samples and to indicate the relative exposures during the arcing of the sample, minute amounts of the salts of thallium, cadmium, cobalt, and beryllium were introduced into the ash samples as controls. Preliminary analyses using these added salts indicated that they did not appreciably alter the concentration of elements present in the samples. Moving-plate studies were made

of some of the samples to obtain an estimate of the quantities of elements present in the samples, the cycles at which they were volatilized, and the relation of these cycles to the cycles at which the elements added for exposure control were volatilized. The same reference standard was used for healthy Valencia orange scions on sweet- and on sour-orange rootstocks. A different reference standard was prepared for Washington Navel orange scions on sweet- and on sour-orange rootstocks. Marsh grapefruit on sweet-orange was referred to the same standard as Marsh grapefruit on sour-orange rootstock. The Eureka lemon scion on sweet-orange rootstock was referred to the same standard as Eureka lemon on Rough-lemon rootstock. The healthy twigs, psorosis-infected trunk woods, and the healthy Valencia orange scion on sour-orange rootstock were all referred to the same standard and are therefore comparable, although the accuracy is lower than for the individual comparisons since the photographic film gamma rating varies slightly from film to film.

The distance between the points at which a spectral line image fades out (L) was measured with a projector comparator employing a ground glass screen. Where L_1 and L_2 are two such values, the intensities of the lines (I_1, I_2) are related to them as indicated in the equation $L_1/L_2 = I_2/I_1$, as pointed out by VANSELOW and LAURANCE (**17**).

Tannin tests were made with $FeCl_3$ on water extracts and on 95 per cent. ethyl-alcohol extracts of the ground wood. The results were consistently negative in all the woods used except that of the twigs; these showed a positive test. Pretreatment with 95 per cent. ethyl alcohol, as recommended for catechol tannins by RITTER and BARBOUR (**13**) was found unnecessary for the trunk-wood samples.

The method of proximate analysis of the various woods was adapted from that used by Goss and PHILLIPS (5) in the "preparation" of the wood for the lignin assay. Our adaptation is described in detail as follows: 1.5-gram samples of the ground wood were weighed into cellulose thimbles, 65 × 22 mm., which were placed in the siphon cups of A. S. T. M. apparatus commonly used in the analysis of rubber products. This apparatus consisted of a block-tin condenser and a 400-ml. Pyrex flask. It was very efficient, losing less solution than the SOXHLET extractors in equal periods of operation. The extraction was made for 6 hours with 150 ml. of 32 parts by weight of 95 per cent. ethyl alcohol and 68 parts by weight of benzene. After extraction, the thimbles were dried in air overnight, and samples were removed to weighing bottles by means of a glass stirring rod; care was taken not to scrape the cellulose loose from the thimble. The weighing bottles with included samples were dried in an oven at 105° C. to remove the last traces of moisture and solvent, cooled in a desiccator for 20 minutes, and weighed. The loss in weight of the samples represented the first group of substances removed and included fats, waxes, resins, and oils.

The second group of substances removed consisted of water-soluble pectins, sugars, and ash. The samples were transferred to 500-ml. Erlenmeyer flasks and boiled with 225 ml. of distilled water under a HOPKINS reflux condenser for 3 hours. The mixture was filtered hot; in order to insure against lowering of the temperature, a jacket of heavy, coiled copper wire was placed around the filtering crucible and heated with a Bunsen flame. The sample was washed thoroughly with hot water.

In the preliminary analyses, sintered-glass crucibles were used. These were replaced with Alundum crucibles (R. A. 98 porosity), however, after it was ascertained that the latter could be used satisfactorily. The Alundum crucible was ignited at a high temperature in a muffle furnace for several hours to oxidize the organic matter. It was then cooled and washed with distilled water, heated for 3 hours in an oven at 105° C., cooled for 20 minutes in a desiccator, and was weighed within 2½ minutes. The same sample was always filtered in the same crucible. When a new sample was used, the crucible was prepared in the manner described; heated for 3 hours at 105° C., cooled for 20 minutes, and reweighed before the new sample was filtered.

The acid-hydrolyzable polysaccharides, consisting of protopectins and hemicelluloses, made up the third group of substances removed from the wood samples. These substances were hydrolyzed with 225 ml. of 1 per cent. HCl by boiling the samples in 500-ml. Erlenmeyer flasks under reflux condensers for a 3-hour period. While still hot, the samples were filtered through the same crucibles used in the previous filtration. The samples were washed with hot distilled water until free of acid, as determined by adding $AgNO_3$ to the wash water. The crucible and contents were dried at 105° C. for 3 hours, cooled in a desiccator for 20 minutes, and weighed.

The fourth group of constituents, which consisted largely of cellulose, was removed from the wood samples by hydrolysis with fuming HCl (sp. gr. 1.210) at 8° C. The method followed was that of Goss and PHILLIPS (**5**) for determining lignin, except that the alcohol-benzene extraction time was limited to 6 hours, as recommended in PHILLIPS's earlier work (**11**).

After the cellulose extraction, the lignin was refluxed in the diluted acid, filtered through the Alundum crucible corresponding to the sample number, and washed until the test water gave a negative test with $AgNO_3$. The lignin was dried at 105° C. for 3 hours, cooled in a desiccator for 20 minutes, and weighed. It was then transferred to a porcelain crucible and ashed at 900° C.

Microanalyses were made on some samples by the KJELDAHL method with the GUNNING-ARNOLD modification. As the amount of nitrogen was small and nearly constant, corrections of the percentage of lignin for nitrogen were not made.

Analyses of the lignin content of citrus woods were also made according to the method of RITTER and BARBOUR (**13**). In this method 72 per cent. H_2SO_4 was employed to remove the cellulose. Hydrolysis was begun with the acid precooled to 20° C. $BaCl_2$ was used in the wash water to test for the complete removal of the SO_4^{--}. Alundum crucibles were employed which necessitated ash corrections.

Results

The results of 25 analyses for the ash of citrus scions, 12 spectrographic analyses for 14 elements in the ash, 28 analyses each for alcohol-benzene extractable substances, water-extractable substances, and 1 per cent. HCl-hydrolyzable substances, 30 analyses for fuming-HCl-hydrolyzable substances, and 54 analyses for lignin are reported. These analyses are based on samples of the trunk wood of scions of eight different healthy trees on three different types of rootstock, four psorosis(virus)-infected scions on one type of rootstock, and twigs from two healthy scions on one type of rootstock. The results reported as percentage of the dry weight of the samples of ground wood are the average of closely agreeing duplicate analyses. The preparation losses are not corrected for ash but the lignin values have been corrected.

Reference to table I shows that in the trunkwoods the healthy wood of Marsh grapefruit scions contained the highest percentage of ash, that the Valencia and Washington Navel orange woods contained an intermediate percentage, while the Eureka lemon wood contained the lowest percentage of ash. The psorosis-infected woods averaged a slightly higher percentage of ash than did healthy Valencia orange trunk wood; those with bark symptoms containing higher percentages of ash than those without. Twigwood (with bark) contained a much higher percentage of ash than did the trunk woods.

Valencia orange, Washington Navel orange, and Marsh grapefruit scions on sour-orange rootstock contained a lower percentage of ash than such scions on sweet-orange rootstock. The Eureka lemon scion on sweet-orange rootstock contained a higher percentage of ash than that on Rough-lemon rootstock. The smaller differences shown in table I are not significant.

Spectrographic analyses of the ash of the citrus woods are shown in table I. The values express ratios of light intensities emitted by the elements in the samples compared. The light intensities recorded are related to the concentration of the elements from which the light is emitted by a function of several variables. The exact relationship for our material and our conditions of excitation were not available and were not determined. Though the line relating concentration and light intensity is probably curvilinear, if points on the curve are selected close together, the relation between the points is approximately linear. In this work the concentra-

TABLE I

PROXIMATE INORGANIC CONSTITUENTS OF WOODS OF HEALTHY AND PSOROSIS-INFECTED CITRUS SCIONS ON VARIOUS ROOTSTOCKS. TOTAL ASH EXPRESSED AS PERCENTAGE OF DRY WEIGHT OF WOOD. APPROXIMATE RELATIVE CONCENTRATION OF ELEMENTS IN ASH OF PAIRS OF WOOD SAMPLES DETERMINED SPECTROGRAPHICALLY AND SHOWN AS THE RATIO OF LIGHT INTENSITIES EMITTED BY ELEMENTS OF ANY TWO WOODS COMPARED, THE VALUE OF THE DENOMINATOR IN EACH RATIO BEING 1

WOOD DESIGNATION	SCION	ROOTSTOCK	PERCENTAGE ASH	WOODS COMPARED	RATIO OF LIGHT INTENSITIES, INDICATING RELATIVE CONCENTRATIONS OF												
					Na	K	Cu	Mg	Ca	Sr	Ba	B	Al	Si	Sn	Pb	Mn	Fe
HEALTHY TRUNK WOOD																		
			%															
a	Valencia orange	Sweet orange	1.53	a/b	0.92	1.00	1.00	1.00	1.15	1.15	0.96	0.77	0.96	1.15	0.16	0.24	1.00	1.15
b	Valencia orange	Sour orange	1.27	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
c	Washington Navel orange	Sweet orange	1.49	c/d	0.80	0.80	1.20	1.00	1.00	1.00	1.10	0.53	0.10	0.32	0.00*/1.00	0.00*/1.00	0.75	0.80
d	Washington Navel orange	Sour orange	1.09	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
e	Marsh grapefruit	Sweet orange	3.24	e/f	1.09	1.09	1.55	1.25	1.14	1.00	1.09	1.25	2.50	2.60	0.00*/1.00	0.51	1.16	1.70
f	Marsh grapefruit	Sour orange	2.40	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
g	Eureka lemon	Sweet orange	1.05	g/h	0.91	1.00	0.72	0.92	1.09	1.00	1.00	1.08	0.91	0.67	0.00*/1.00	1.00	0.50	0.45
h	Eureka lemon	Rough lemon	0.65	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
PSOROSIS-INFECTED TRUNK WOOD (WITH BARK SYMPTOMS)																		
i	Valencia orange Scion 1	Sour orange	1.64	i/b	0.92	1.12	1.00	1.15	1.20	1.00	1.05	0.70	0.63	0.80	0.51	0.55	0.91	1.00
	Scion 2	Sour orange	1.77	i/j	0.82	1.12	0.94	0.80	0.91	0.80	1.30	0.87	1.60	1.04	1.33	1.20	1.00	1.00
PSOROSIS-INFECTED TRUNK WOOD (WITH NO BARK SYMPTOMS)																		
j	Valencia orange Scion 1	Sour orange	1.52	j/b	1.00	1.00	1.00	1.33	1.18	1.20	0.80	0.75	0.45	0.79	0.33	0.52	1.00	1.00
	Scion 2	Sour orange	1.13	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
HEALTHY TWIG WOOD (WITH BARK)																		
k	Valencia orange (2 scions)	Sour orange	5.97	k/b	0.63	0.88	1.00	1.20	1.37	1.10	1.13	0.74	0.86	0.93	0.33	0.57	0.86	0.60

* Ratios are shown, as the quotients would be meaningless. The ratios show that in scions on sweet-orange rootstocks tin was not detected, but in the scions on sour-orange rootstock tin was detected. Lead was not detected in Washington Navel orange scion on sweet-orange rootstock but was detected in this scion on sour-orange rootstock.

tion of elements was not greatly different. The ratio of the element concentration to the light intensity was, therefore, approximately linear. The light intensity values recorded are reciprocals of the lengths of the blank spaces between the emission lines. Thus a value lower than 1 indicates that the concentration of the element in the numerator is smaller than that in the denominator; and a value greater than 1, vice versa.

The results are probably accurate to ± 10 per cent. Thus, values differing by 20 per cent. or more will be considered significantly different. Comparisons are valid only for single elements between pairs of samples, as listed, except where several samples are referred to a common reference sample, as in the cases of comparisons *a* and *b*, *i* and *b*, *j* and *b*, *k* and *b* (table I), where it is possible to make comparisons between *a*, *i*, *j*, and *k*. In these cases the accuracy is less, because of variation in film gamma, and differences of 25 per cent. will be considered significantly different.

The ash samples from the healthy trunk wood of Washington Navel orange scion on sweet-orange rootstock (*c*) and Eureka lemon scion on Rough-lemon rootstock (*h*) were so small that the results could not be checked by duplicate determinations as in all of the other analyses. The shortage of material in these two samples made it necessary to use a compromise in exposure conditions in order to estimate the concentrations of all the elements in question in a single exposure.

The trunk-wood ash of healthy Valencia orange scion on sweet-orange rootstock contained a lower concentration of boron, tin, and lead than did the Valencia orange scion on sour-orange rootstock. Washington Navel scion on sweet-orange rootstock contained a lower concentration of sodium, potassium, boron, aluminum, silicon, manganese, tin, lead, and iron, and a higher concentration of copper than did Washington Navel orange scion on sour-orange rootstock. The internal standard applicable to aluminum and silicon shows low light intensities indicating that the light intensity values are low for this sample. Marsh grapefruit scion on sweet-orange rootstock contained a lower concentration of tin and lead and a higher concentration of copper, magnesium, boron, aluminum, silicon, and iron than Marsh grapefruit scion on sour-orange rootstock. Eureka lemon scion on sweet-orange rootstock contained a lower concentration of copper, silicon, manganese, and iron than Eureka lemon scion on Rough-lemon rootstock, as shown in table I.

The trunk-wood ash of psorosis-infected Valencia orange scion on sour-orange rootstock, having bark symptoms, contained lower concentrations of boron, aluminum, silicon, tin, and lead, and higher concentrations of calcium than the trunk-wood ash of healthy Valencia orange scion on sour-orange rootstock. Psorosis-infected Valencia orange scion having bark symptoms contained a lower concentration of magnesium and strontium, and

on sour-orange rootstock tin was detected. Lead was not detected in Washington Navel orange scion on sweet-orange rootstock but was detected in this scion on sour-orange rootstock.

a higher concentration of barium, aluminum, tin, and lead than psorosis-infected Valencia orange scion not having bark symptoms. The psorosis-infected Valencia orange scion not having bark symptoms contained a lower concentration of barium, boron, aluminum, silicon, tin, and lead, and a higher concentration of magnesium and strontium than healthy Valencia orange scion on sour-orange roostock.

Twig wood (with bark) of healthy Valencia orange scion on sour-orange rootstock contained a lower concentration of sodium, boron, tin, lead, and

TABLE II

PROXIMATE ORGANIC CONSTITUENTS OF WOODS OF HEALTHY AND PSOROSIS-INFECTED CITRUS SCIONS ON VARIOUS ROOTSTOCKS, DETERMINED AS PREPARATION LOSSES FOR THE PHILLIPS HCl METHOD FOR LIGNIN AND EXPRESSED AS PERCENTAGE OF DRY WEIGHT. VALUES ARE MEANS OF DUPLICATE ANALYSES

Scion	Rootstock	Percentage loss through: Alcohol-benzene extraction	Water extraction	1 per cent. HCl hydrolysis	Fuming HCl hydrolysis
Healthy trunk wood					
		%	%	%	%
Valencia orange	Sweet-orange	5.64	12.07	32.28	35.74
Valencia orange	Sour-orange	8.43	12.14	32.97	33.51
Washington Navel orange	Sweet-orange	4.09	4.33	34.51	40.80
Washington Navel orange	Sour-orange	5.19	7.12	33.17	37.49
Marsh grapefruit	Sweet-orange	4.15	4.96	35.75	38.84
Marsh grapefruit	Sour-orange	7.04	8.67	36.91	31.60
Eureka lemon	Sweet-orange	4.73	5.94	34.09	38.77
Eureka lemon	Rough-lemon	3.57	4.70	30.99	43.04
Psorosis-infected trunk wood (with bark symptoms)					
Valencia orange					
Scion 1	Sour-orange	7.48	12.80	35.17	31.85
Scion 2	Sour-orange	5.46	10.63	35.99	32.30
Average		6.47	11.71	35.58	32.07
Psorosis-infected trunk wood (with no bark symptoms)					
Valencia orange					
Scion 1	Sour-orange	4.87	13.83	36.86	32.62
Scion 2	Sour-orange	5.18	10.97	34.16	34.51
Average		5.02	12.40	35.51	33.56
Healthy twig wood (with bark)					
Valencia orange					
Scion 1	Sour-orange	12.96	13.78	32.93	28.61
Scion 1	Sour-orange	.....	.....	.....	29.92
Scion 2	Sour-orange	11.50	9.54	31.81	34.02
Average		12.23	11.66	32.37	30.85

iron, and a higher concentration of magnesium and calcium than healthy Valencia orange scion trunk wood on sour-orange rootstock.

Percentage loss in the alcohol-benzene extraction represents the aggregate amount of fats, waxes, resins, and oils in the various citrus woods. Reference to table II shows that in this extraction the percentage losses from the sour-orange rootstocks (8.43, 5.19, and 7.04) were higher than those from the sweet-orange rootstocks (5.64, 4.09, and 4.15) and that the sour-orange rootstocks therefore produced higher percentages of these substances than the sweet-orange rootstocks. Washington Navel orange on sour-orange rootstock yielded 1.10 per cent. more alcohol-benzene extractable constituents than Washington Navel orange on sweet-orange rootstock. Valencia orange on sour-orange rootstock yielded 2.79 per cent. more alcohol-benzene extractable constituents than Valencia orange on sweet-orange rootstock, and Marsh grapefruit on sour-orange rootstock yielded 2.89 per cent. more alcohol-benzene extractable constituents than Marsh grapefruit on sweet-orange rootstock.

Percentage loss in the hot water extraction (table II) represents the aggregate amount of water-soluble pectins, sugars, and ash in the various woods. In this extraction, the percentage losses of the scions on sour-orange rootstocks (12.14, 7.12, and 8.67) ranged from slightly higher to nearly double those of the sweet-orange rootstocks (12.07, 4.33, and 4.96). The Valencia orange scion was affected least by the rootstock, while the Marsh grapefruit was affected most.

Acid-hydrolyzable polysaccharides, as protopectins and hemicelluloses, are represented in the percentage loss on 1 per cent. HCl hydrolysis (table II). No regular trend was effected by the type of rootstock. Valencia orange and Marsh grapefruit showed slightly greater percentage losses of acid-hydrolyzable substances on sour-orange rootstock (32.97, and 36.91) than on sweet-orange (32.28, and 35.75); the Washington Navel orange showed a lower percentage on sour (33.17) than on sweet (34.51). In comparison, the small value representing the percentage loss (principally hemicelluloses) in the Eureka lemon on Rough lemon (30.99) is notable.

Cellulose is the main constituent represented by the loss in hydrolysis with fuming HCl. In this extraction, Valencia orange, Washington Navel orange, and Marsh grapefruit scions on sour-orange rootstock show smaller percentage losses of this cell-wall material (33.51, 37.49, and 31.60) than such scions on sweet-orange rootstock [(35.74, 40.80, and 38.84) (table II)]. It is interesting to note not only the very high percentage of cellulose found in Eureka lemon on Rough-lemon rootstock (43.04), as compared with that found in Eureka lemon on sweet-orange rootstock (38.77), but also this high value as compared with most of the other values obtained (table II). The high percentage of cellulose may be of especial significance. DORE (**4**) has

discussed critically the proximate analysis of woods and should be consulted as to its limitations.

The percentage of lignin determined by the PHILLIPS HCl method (table III) was higher for Marsh grapefruit on sweet-orange rootstock (16.24) than on sour (15.65). A larger percentage of lignin was found in

TABLE III

LIGNIN CONTENT OF WOODS OF HEALTHY AND OF PSOROSIS-INFECTED CITRUS SCIONS ON VARIOUS ROOTSTOCKS, DETERMINED BY THE PHILLIPS HCl AND BY THE RITTER H_2SO_4 METHODS AND EXPRESSED AS PERCENTAGE OF DRY WEIGHT, CORRECTED FOR ASH. VALUES ARE MEANS OF DUPLICATE ANALYSES

SCION	ROOTSTOCK	LIGNIN DETERMINATION	
		HCl METHOD	H_2SO_4 METHOD
HEALTHY TRUNK WOOD			
		%	%
Valencia orange	Sweet-orange	12.84	12.99
Valencia orange	Sour-orange	12.65	14.02
Average		12.74	13.50
Washington Navel orange	Sweet-orange	16.13	21.36
Washington Navel orange	Sour-orange	16.89	20.69
Average		16.51	21.02
Marsh grapefruit	Sweet-orange	16.24	21.27
Marsh grapefruit	Sour-orange	15.65	21.37
Average		15.94	21.32
Eureka lemon	Sweet-orange	16.18	21.82
Eureka lemon	Rough-lemon	17.35	20.96
Average		16.76	21.39
PSOROSIS-INFECTED TRUNK WOOD (WITH BARK SYMPTOMS)			
Valencia orange			
Scion 1	Sour-orange	11.60	14.67
Scion 2	Sour-orange	12.94	14.90
Average		12.27	14.78
PSOROSIS-INFECTED TRUNK WOOD (WITH NO BARK SYMPTOMS)			
Valencia orange			
Scion 1	Sour-orange	11.43	16.86
Scion 2	Sour-orange	13.46	17.70
Average		12.44	17.28
HEALTHY TWIG WOOD (WITH BARK)			
Valencia orange			
Scion 1	Sour-orange	11.55	.....
Scion 1	Sour-orange	11.51	.....
Scion 2	Sour-orange	12.80	.....
Average		11.95	.....

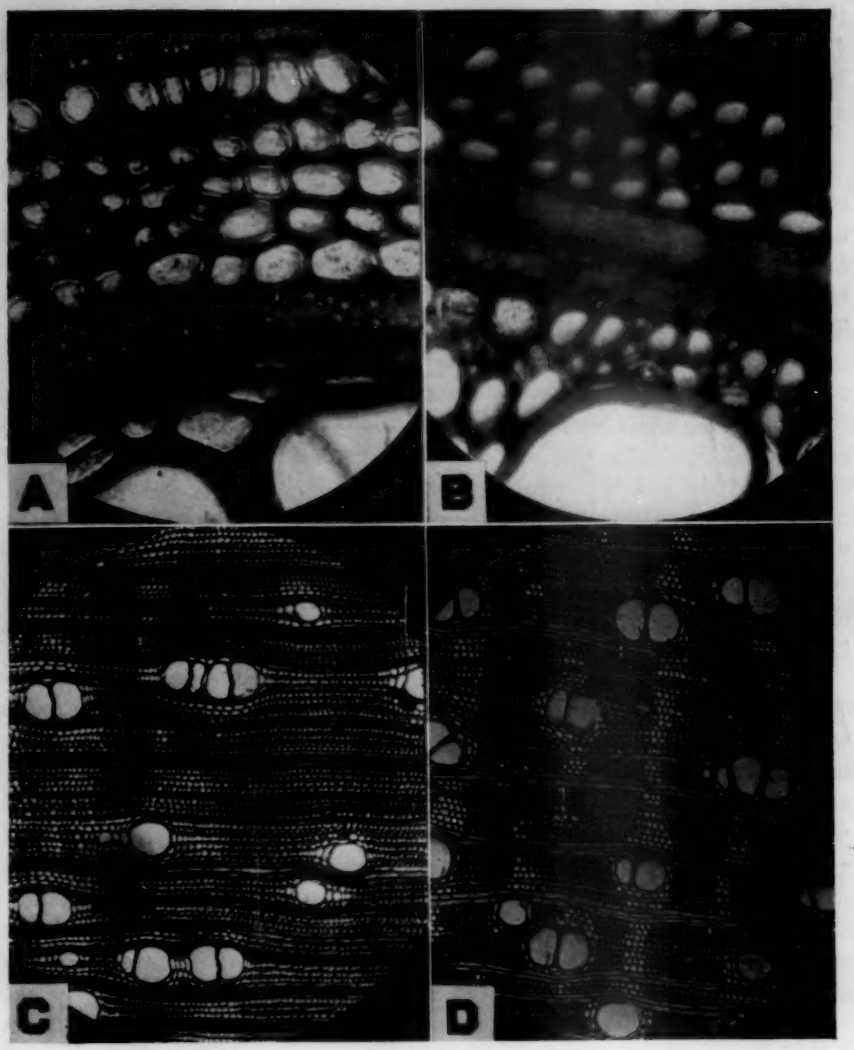

FIG. 2. A. Transverse section of Valencia orange wood showing the thick cell walls (fibers, 5.1 μ in width; tracheids, 3.2 μ; vessels, 4.8 μ). The walls are composed of a large portion of clear unstained material (higher carbohydrate) and a small portion of safranin stained material (lignin). (×518.) B. Transverse section of Washington Navel orange wood showing comparatively thin cell walls (fibers, 4.5 μ in width; tracheids, 2.2 μ; vessels, 5.8 μ). The walls show almost no material unstained with safranin. (×518). Transverse sections of Valencia orange wood (C) and of Washington Navel orange wood (D.) (Both ×57.) Because of the smaller proportion of fibers, the wood of the former is more porous than that of the latter.

the Washington Navel orange on sour-orange rootstock (16.89) than on sweet (16.13). The high percentage of lignin in the Eureka lemon on Rough-lemon rootstock (17.35), as compared with the percentage of lignin in the Eureka lemon on sweet-orange rootstock (16.18) or as compared with the other lignin values, is notable. The percentage of lignin was lower in Valencia orange wood than in the other citrus woods analyzed. Microscopic examination of sections of Valencia orange and Washington Navel orange woods shows that the cell walls of the former are thicker than those of the latter, but the cell walls of the Valencia orange do not show such wide bands of safranin stain (fig. 2) as those of the Washington Navel orange. This suggests that the Valencia wood contains less lignin.

Assay of lignin by the RITTER H_2SO_4 method (table III) shows a relatively larger value in every instance in which the HCl method shows a smaller value, when rootstocks are compared. Comparison of mean percentages of lignin for woods of the different varieties and species, however, shows that the general trend is practically the same, whichever method is used. The one exception is in the case of the Washington Navel orange wood, which shows a higher percentage of lignin (16.51) than Marsh grapefruit wood (15.94) when the HCl method is used, but a lower percentage of lignin (21.02) than Marsh grapefruit wood (21.32) when the H_2SO_4 method is used.

Valencia orange wood (scion) from psorosis-infected trees grown on sour-orange rootstock, when analyzed, gave more variable results than the healthy woods. This variation is somewhat obscured in the results reported in table II, since these are averages of duplicate analyses. Alcohol-benzene extractable materials composed a higher percentage of the dry weight of the wood of those scions having bark symptoms of the disease than of those having no bark symptoms, but this difference is not significant. No clear trend of the proximate constituents was evident in the water extraction or in the 1 per cent. HCl hydrolysis. The percentage of materials hydrolyzed with fuming HCl was slightly lower, however, in the scions with bark symptoms, but this difference is not significant.

Psorosis-infected woods of scions with or without bark symptoms contained lower percentages of alcohol-benzene extractable substances and higher percentages of 1 per cent. HCl-hydrolyzable substances than healthy Valencia scions. Scions with bark symptoms contained less fuming-HCl-hydrolyzable material than healthy scions. No clear trend was noted for the water-extractable materials.

In all cases, duplicate samples of wood from scions having psorosis symptoms in the bark varied from 2 to 3 per cent. in lignin content, as determined by the PHILLIPS HCl and by the RITTER H_2SO_4 methods. One set of samples from infected scions showing no bark symptoms gave similar

TABLE IV

F AND t VALUES FOR COMPARISONS TESTED FOR SIGNIFICANCE OF DIFFERENCE BY ANALYSIS OF VARIANCE AND "t" TEST

COMPARISONS	F	t
PERCENTAGE OF ASH (HEALTHY WOOD)		
	%	%
Marsh grapefruit, both rootstocks *vs.* Valencia orange, both rootstocks	25.605‡	.....
Valencia orange, both rootstocks *vs.* Eureka lemon, both rootstocks	13.023*	.....
Washington Navel orange, sweet-orange rootstock *vs.* Washington Navel orange sour-orange rootstock	.....	.....
Marsh grapefruit, sweet-orange rootstock *vs.* Marsh grapefruit, sour-orange rootstock	8.301†	27.184‡
Valencia orange, sweet-orange rootstock *vs.* Valencia orange, sour-orange rootstock	75.111*	.....
Eureka lemon, sweet-orange rootstock *vs.* Eureka lemon, Rough-lemon rootstock	5.861†	10.256‡
PERCENTAGE OF ASH (DISEASED WOOD)		
Psorosis-infected Valencia orange (bark symptoms), sour-orange rootstock *vs.* healthy Valencia orange, sour-orange rootstock	12.620*	.....
Psorosis-infected Valencia orange (no bark symptoms), sour-orange rootstock *vs.* healthy Valencia orange, sour-orange rootstock	0.000†	1.795†
Psorosis-infected Valencia orange (bark symptoms), sour-orange rootstock *vs.* psorosis-infected Valencia orange, sour-orange rootstock (no bark symptoms)	9.471*	.....
Valencia orange, sour-orange rootstock trunk wood *vs.* Valencia orange, sour-orange rootstock, twig wood	57.179‡	.....
PERCENTAGE OF ORGANIC CONSTITUENTS (HEALTHY WOOD)		
Alcohol-benzene soluble constituents Valencia orange, Washington Navel orange, Marsh grapefruit, sweet-orange rootstock *vs.* sour-orange rootstock	10.523‡	.....
Water-soluble constituents Valencia orange, Washington Navel orange, Marsh grapefruit, sweet-orange rootstock *vs.* sour-orange rootstock	1.312†	15.089‡
Dilute HCl hydrolyzable constituents Eureka lemon, sweet-orange rootstock *vs.* Eureka lemon, Rough-lemon rootstock	61.170*	.....
Fuming HCl hydrolyzable constituents Valencia orange, Washington Navel orange, Marsh grapefruit, sweet-orange rootstock *vs.* sour-orange rootstock	8.444*	.....
Fuming HCl hydrolyzable constituents Eureka lemon, sweet-orange rootstock *vs.* Rough-lemon rootstock	14.532†	21.616‡
Fuming HCl hydrolyzable constituents Eureka lemon, Rough-lemon rootstock *vs.* Valencia orange, Washington Navel orange, Marsh grapefruit, Eureka lemon on sweet-orange rootstock	7.541*	.....
Lignin Valencia orange, sweet-orange rootstock *vs.* sour-orange rootstock	0.696†	1.348†
Lignin Washington Navel orange, sweet-orange rootstock *vs.* sour-orange rootstock	23.385*	.....
Lignin Eureka lemon, sweet-orange rootstock *vs.* Rough-lemon rootstock	8.052†	8.298‡
Lignin Marsh grapefruit, sweet-orange rootstock *vs.* sour-orange rootstock	8.681†	4.184‡
Lignin Valencia orange, both rootstocks *vs.* Washington Navel orange, Marsh grapefruit, Eureka lemon, both rootstocks (lemon on sweet only)	130.205‡	.....
Lignin all scions on all rootstocks, HCl method *vs.* all scions on all rootstocks, HCl method	86.714‡	.....
Lignin all scions on all rootstocks, H_2SO_4 method *vs.* all scions on all rootstocks H_2SO_4 method	28.655‡	.....

TABLE IV—(*Continued*)

Comparisons	F	t
Percentage of organic constituents (diseased wood)		
	%	%
Alcohol-benzene soluble constituents psorosis-infected Valencia orange, bark symptoms *vs.* no bark symptoms	0.010†	0.794†
Alcohol-benzene soluble constituents all psorosis-infected Valencia orange, sour-orange rootstock *vs.* Valencia orange healthy, sour-orange rootstock	17.263‡	.....
Dilute HCl hydrolyzable constituents all psorosis-infected Valencia orange, sour-orange rootstock *vs.* Valencia orange healthy, sour-orange rootstock	13.120‡	.....
Fuming HCl hydrolyzable constituents psorosis-infected Valencia orange, bark symptoms *vs.* no bark symptoms	2.940†	1.869†
Alcohol-benzene soluble constituents Valencia orange (trunkwood), sour-orange rootstock *vs.* Valencia orange (twigwood), sour-orange rootstock	27.289‡	.....
Lignin Valencia orange (trunkwood) sour-orange rootstock *vs.* Valencia orange (twigwood), sour-orange rootstock	7.245*	.....
Lignin, all scions, all rootstocks, HCl method *vs.* all scions, all rootstocks, H_2SO_4 method	15.597‡	.....

‡ Highly significant; * Significant; † Not significant.

variability with the two methods of assay, while a second set of samples gave consistent results with both methods. The averages of the percentages of lignin in each scion in the psorosis (virus)-infected Valencia wood (table III) agree fairly well with those found for healthy wood (table III) except in the case of wood samples from scions showing no bark symptoms, assayed by the Ritter. H_2SO_4 method. The average percentage of lignin in these samples is 3.2 per cent. higher than that of the healthy Valencia wood assayed by the same method. In all analyses (table III), the Ritter H_2SO_4 method yielded higher percentages of lignin than the Phillips HCl method.

Analysis showed that twig wood (including bark) from healthy Valencia orange scions on sour-orange rootstock (table II) contained a higher percentage of alcohol-benzene extractable substances than trunk wood. There is no clear trend in the substances lost in water extraction, in hydrolysis with 1 per cent. HCl, or in hydrolysis with fuming HCl.

The lignin analyses of twigs of Valencia orange scions on sour-orange rootstock are also shown in table III. The mean percentage of lignin in the twigs, as determined by the Phillips HCl method, is 11.95. This value is 0.70 per cent. lower than the mean of similar assays by the Phillips HCl method of the trunk-wood samples taken just above the bud union (table III).

Discussion and conclusions

Oliveri and Guerrieri (**9**), working in Italy, found that green, tender orange wood contained 40.99 grams of ash per 1,000 parts (4.10 per cent.);

old, hard orange wood contained 43.89 grams per 1,000 parts (4.39 per cent.). Green, tender lemon wood was found to contain 41.94 grams per 1,000 parts (4.19 per cent.), while old, hard lemon wood contained 45.68 grams per 1,000 parts (4.57 per cent.). The authors do not state the variety of orange or lemon used, the age of the trees, the exact portions of the tree from which the old, hard wood was obtained, nor whether bark was included. The fact that the percentage of ash for the old, hard orange wood is of approximately the same magnitude as our values for twigs with bark suggests that old twigs were used for analysis.

BLAIR (**2**), working in Florida, reported that twigs of very young (late) Valencia orange scions on sour-orange rootstock (trees in the field, 1909 to 1910) assayed from 3.83 to 4.57 per cent. ash. These values are slightly lower than the values we have found for Valencia orange twigs on older trees in California. Since neither BLAIR nor OLIVERI and GUERRIERI stated the length of time the wood was dried or the length of time the samples remained in the desiccator, it is impossible to say whether the differences noted in our results are actual differences in the ash content or merely differences due to the higher water content of their samples. Citrus woods are extremely hygroscopic, a 4- or 5-gram sample absorbing as much as 200 mg. of moisture from a $CaCl_2$-charged desiccator in 24 hours if the wood is thoroughly dried.

The differences in habit of growth exhibited by citrus scions and fruit of various species and varieties on different rootstocks, generally observed in the field, are a reflection of the different inorganic and organic constitution of the scions. Various portions of the same scion have different percentage compositions, as shown by comparing twig and trunk samples. This suggests that a virus disease also affects the percentage composition of the scions.

Valencia orange, Washington Navel orange, and Marsh grapefruit scions on sweet-orange rootstocks accumulated a higher percentage of ash in the trunk wood than did these scions on sour-orange rootstocks. The Eureka lemon scion on sweet-orange rootstock accumulated significantly more ash in the wood than did Eureka lemon on Rough-lemon rootstock.

Psorosis-infected scions with bark symptoms contained a slightly higher percentage of ash than did trunk wood of healthy Valencia orange scions. Infected scions with no bark symptoms gave inconclusive results, one scion assaying a higher percentage and one a lower percentage of ash than the healthy scions. Twig wood (with bark) had a higher percentage of ash than trunk wood of Valencia orange scions.

While the scion-rootstock combinations showed, in a general way, approximately the same relative concentration of elements in the ash, in some cases it seemed that there may have been selective absorption of elements, as in-

dicated by higher accumulations of certain metals. The more marked instances are the following: the lower accumulation of tin and lead by healthy Valencia orange scion on sweet-orange rootstock than by the same scion on sour-orange rootstock; the lower accumulation of aluminum, silicon, tin, and lead by Washington Navel orange scion on sweet-orange rootstock than by the same scion on sour-orange rootstock; the higher accumulation of aluminum and silicon and lower accumulation of tin and lead by Marsh grapefruit scion on sweet-orange rootstock than by the same scion on sour-orange rootstock; and the lower accumulation of manganese and iron by Eureka lemon scion on sweet-orange rootstock than by the same scion on Rough-lemon rootstock. In each of these cases one scion-rootstock combination contained elements in a concentration two or more times that of the one with which it has been compared. Though every precaution was taken to eliminate contamination of the samples with dust, it is possible that these differences may be due to such contamination or to traces of metals accumulated in grinding.

Psorosis-infected trunk wood of Valencia orange scion on sour-orange rootstock, where bark symptoms were visible, showed relatively low accumulations of boron, aluminum, silicon, tin, and lead, as compared with the ash of a healthy scion of the same kind on a similar rootstock. This comparison is closely approximated in the psorosis-infected Valencia orange scion having no visible bark symptoms, indicating that the accumulation of inorganic ions is probably related to the general vigor of the tree.

While healthy twig wood (with bark) showed concentrations of several elements in the ash to be different from those in the ash of healthy trunk wood of Valencia orange scion on sour-orange rootstock, tin was the element which was present in the lowest relative concentration. The higher concentration of magnesium in the twig sample may be explained by the chlorophyll content of the bark of the twig.

The various citrus scions on sour-orange rootstock gave higher percentages of alcohol-benzene extractable materials, and higher percentages of water-extractable substances than the citrus scions on sweet-orange rootstock.

Extractions with dilute (1 per cent.) HCl indicated that substances hydrolyzed by HCl were not closely related to the type of rootstocks, since no clear trend was noted for the various citrus scions. The scions on sweet-orange rootstock, however, gave higher percentages of substances removable by the fuming HCl (probably largely cellulose) than those on sour-orange rootstock.

All percentages of lignin reported are corrected for ash since a spectrographic analysis (fig. 3) showed that a large percentage of ash in the lignin was aluminum contributed by the Alundum crucibles. The strikingly heavy

lines in the spectrogram (fig. 3, A) are aluminum lines indicating the presence of an amount of aluminum far in excess of the aluminum normally present in citrus woods.

The mean error in the determination of lignin, in healthy woods by the PHILLIPS HCl method was 0.31 per cent.; that in the determination by the RITTER H_2SO_4 method, 0.80 per cent. An analysis of variance showed that the individual percentages of lignin obtained by either the PHILLIPS or the RITTER method, and recorded for each species or variety, are significantly different. How much individual tree variability may affect these results cannot be accurately estimated at the present time, although comparison of analyses of two of the psorosis-infected trees (table III) suggests that the variability may be as much as 2 per cent.

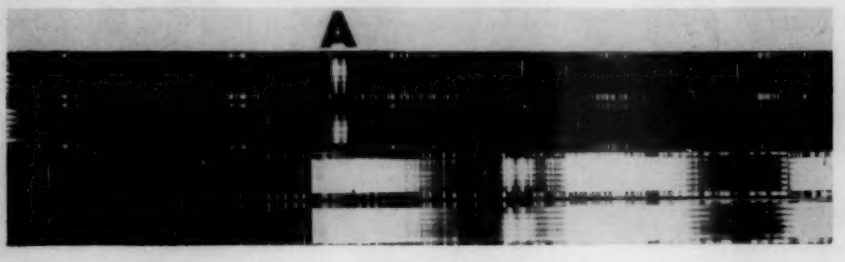

FIG. 3. Arc spectrograms of the ashes of lignin obtained by analyses with the HCl method (upper row of each pair) and with the H_2SO_4 method (lower row of each pair), showing the intense aluminum lines at A.

CARYL (3) has pointed out that 48 strains and variations of the Washington Navel orange, 47 of the Valencia orange, 9 of the Marsh grapefruit, and 12 of the Eureka lemon are now grown in California. There are also wide variations in soil, water, and climatic factors, as well as in cultural practices. Data on which to base an opinion of the effect of these factors on the percentages of constituents of citrus woods are not available; we feel, however, that they very likely do have an effect. When, in addition, the widely different areas in which citrus is grown in California are considered, the problem of adequate sampling as a basis of generalization is at once evident.

In the Riverside area, the Washington Navel orange scions on sweet- and on sour-orange rootstocks have a tendency toward greater cellulose formation and lignification than do Valencia orange scions on similar rootstocks. Eureka lemon scions on sweet-orange and Rough-lemon rootstocks and Marsh grapefruit scions on sweet-orange rootstocks also have a tendency to produce greater percentages of cellulose and lignin than the Valencia orange scions on sweet and sour rootstocks. Marsh grapefruit scions on sour-orange rootstock produce less cellulose than Valencia orange on sweet or sour root-

stocks; on either rootstock, however, they produce a higher percentage of lignin than do Valencia orange scions on either rootstock. Except for the lower production of cellulose by the Marsh grapefruit scions on sour-orange rootstock, these comparisons suggest that Valencia orange is a relatively low producer of cellulose and lignin.

The Washington Navel orange in the Riverside area sets its fruit in the spring, and the mature fruit is generally picked before a new crop is set. This variety matures its fruit rapidly, and if the fruit is allowed to remain on the tree for a few months after the new crop is set, it is occasionally granulated; that is, the sap gelates and the cell walls thicken and lignify. In California, granulation in navel oranges is not a matter for serious consideration, but in some countries the fruit of this variety granulates so badly that it cannot be grown for commercial purposes, especially on certain rootstocks. The Valencia variety also sets its fruit in the spring but matures the fruit so slowly that a second crop is set and fairly well developed before the first is matured. The Washington Navel fruit is matured in about 10 months, and the Valencia fruit in about 13 months, although there are wide fluctuations in both cases. If the Valencia crop is not picked until late (latter part of September) it is often more or less granulated. This suggests a positive correlation between rate of fruit maturation and granulation (excessive production of cellulose and lignin in the fruit) and the tendency toward cellulose and lignin production of the stem.

It is also known that Valencia orange scions on Rough-lemon rootstocks are among the high producers of granulated fruit (**1**). Unfortunately, samples of Valencia orange wood (scions) on Rough-lemon rootstock could not be obtained, but since Eureka lemon on Rough-lemon rootstock produced a high percentage of cellulose and of lignin, it may be surmised that this might prove true of Valencia orange on Rough lemon also. Thus there is a suggestion in this comparison that production of high percentages of granuated fruit by Valencia orange scions on Rough-lemon rootstock may be found to be the result of a tendency of scion and rootstock combination to produce relatively high percentages of lignin in the trunk wood.

Some citrus observers have maintained that the problem of granulation is one of overmature fruit. This correlates with the general botanical observation that lignification of parenchymatous tissue may accompany maturation and that sclerenchymatous tissues contain a higher percentage of lignin at maturity. Lignification has been observed by Wilson (**19**) to occur in mature phloem of several types of plants, and such a process may well occur in the thin-walled cells of "overmature" citrus fruits.

Haas and Klotz (**6**) showed that granulated Valencia oranges contained 6.13 per cent. ash, as compared with 5.16 per cent. in normal fruits, when the results were expressed as percentage of dry matter. The ash content

of wood of trees producing large percentages of granulated fruit might therefore be expected to be high.

In this connection it is of interest to note that the percentage of ash of the Eureka lemon scion on Rough-lemon rootstock is significantly lower than that of any of the other woods assayed, including Marsh grapefruit. HAAS and KLOTZ (**6**) showed, however, that Eureka lemon fruit pulp contained 4.61 per cent. ash, whereas Marsh grapefruit pulp averaged 3.37 per cent. This suggests that trees which accumulate little ash in the trunk wood may accumulate more ash in the fruits than trees accumulating larger amounts of ash in the wood, and that such a condition may be correlated with the high percentage of granulated fruit produced by Valencia orange scions on Rough-lemon rootstock.

While some environmental factors are known to accelerate the maturation of plants, the factors which influence lignification are not well known, nor are the factors which accelerate granulation in citrus fruits.

The irregular results obtained in the analysis of psorosis-infected wood are, we believe, due to the ineffectual removal of gums shown by WEBBER and FAWCETT (**18**) to be formed in the vessels of the wood in this disease.

Psorosis evidently affects the normal metabolism of the Valencia scion. The diseased scions, both those with and those without bark symptoms, contained less alcohol-benzene extractable material and larger quantities of 1 per cent. HCl-hydrolyzable substances than healthy scions. The diseased wood having bark symptoms contained a lower percentage of fuming-HCl-hydrolyzable material. Water-extractable substances and lignin were not greatly affected by the presence of the virus.

Twig samples from the two scions of Valencia orange on sour-orange rootstock showed considerable variation. These samples are not strictly comparable to samples of the trunk wood since the bark was included in the former; but outstanding differences seem to be the higher percentage of ash and the higher percentage of alcohol-benzene extractable materials in the twig samples.

Comparisons of the results have been treated by analysis of variance in order to properly appraise the significance of such comparisons. In a number of cases the number of degrees of freedom was small; thus the analysis of variance indicated that the difference was not significant. The "t" test was applied in these cases in order to more critically judge the significance of the difference. In applying the "t" test, it was essential to calculate the standard errors of the various analyses. The standard errors in per cent. were: ash, 0.039; alcohol benzene soluble constituents, 0.214; water soluble constituents, 0.145; dilute HCl hydrolyzable constituents, 0.239; concentrated HCl hydrolyzable constituents, 0.198; lignin, 0.141. The various

comparisons made in this paper are listed in table IV with the "F" and "t" values and their significance rating.

Our results, obtained by the PHILLIPS HCl and the RITTER H_2SO_4 methods, were different and accord with the findings of other investigators. The difference is perhaps due to the action of H_2SO_4 on certain sugars such as xylose, fructose, arabinose (to a small extent), and polysaccharides containing pentose sugars which form an insoluble residue with 72 per cent. H_2SO_4 and increase the apparent lignin percentage (**8**). NORMAN (**7**) also showed that aldehydes, phenols, and nitrogenous substances of various types condense with 72 per cent. H_2SO_4 to give high apparent lignin values. PHILLIPS and Goss (**12**) have made comparative studies on the HCl method and other methods of determining lignin. They have listed factors affecting the determination of lignin by the HCl method, as RITTER, SEBORG, and MITCHELL (**14**) and SHERRARD and HARRIS (**15**) have done for the H_2SO_4 method.

It is not in the province of this paper to reappraise the methods, but we would call attention to the fact that in our hands the error between duplicates was smaller with the HCl method; therefore, we have felt that it was more reliable. What the lignin values obtained by the two methods represent, however, is difficult to explain. For example, a sample of Aspen lignin prepared by the H_2SO_4 method in another laboratory was rerun by us by the PHILLIPS HCl and the RITTER H_2SO_4 methods. The preparation losses were approximately 25 per cent. and 20 per cent., and the yields of lignin approximately 65 per cent. and 80 per cent., respectively. There was a considerably larger loss of the apparent lignin value in the hydrolysis with fuming HCl than would be expected from the differences in assay by the two methods.

Summary

1. Lignin analyses were made on the sapwood of Washington Navel orange, Valencia orange, Eureka lemon, and Marsh grapefruit scions on sweet- and on sour-orange rootstocks, using the PHILLIPS HCl and the RITTER H_2SO_4 methods.

2. The proximate constituents represented by extraction losses both in alcohol-benzene and in hot water were higher for all the scions on sour-orange than for those on sweet-orange rootstocks and indicated higher percentages of oil and fatty substances, sugars, and pectins.

3. The percentage of constituents hydrolyzed by dilute (1 per cent.) HCl was lowest in wood from the Eureka lemon scion on Rough-lemon rootstock and highest in wood from the Marsh grapefruit scion on sour-orange rootstock. Valencia orange scions contained lower percentages of these con-

stituents than Washington Navel orange scions on either sweet- or sour-orange rootstocks.

4. The percentage of constituents hydrolyzed by fuming HCl was lowest in wood from the Marsh grapefruit scion on sour-orange rootstock and highest in wood from the Eureka lemon scion on Rough-lemon rootstock. Valencia orange scions contained lower percentages of these constituents than Washington Navel orange scions on either sweet- or sour-orange rootstocks.

5. The lignin content of various species and varieties of healthy citrus woods on various rootstocks assayed from 12.65 to 17.35 per cent. of the dry weight of the wood by the PHILLIPS HCl method and from 12.99 to 21.82 per cent. by the RITTER H_2SO_4 method.

6. Valencia orange scions contained the least lignin; Eureka lemon scions on Rough-lemon rootstock, the most. Psorosis-infected wood gave rather irregular results, although the average lignin content of this wood was about the same as that of healthy wood. Percentages of other constituents of the diseased wood differed from those of healthy wood.

7. Significantly higher values were obtained with the RITTER H_2SO_4 method than with the PHILLIPS HCl method.

8. The percentage of ash of the wood of healthy scions on sweet-orange rootstocks was higher than that of healthy scions on sour-orange rootstocks. Eureka lemon on Rough-lemon rootstock had the lowest percentage of ash. Psorosis-infected woods with bark symptoms had a slightly higher percentage of ash than healthy woods. Twig wood (with bark) contained a higher percentage of ash than trunk wood.

9. Spectrographic analyses of the ash of wood of healthy citrus scions of a given species showed that, in general, these scions contained about the same relative concentration of elements, whether on sweet-, sour-orange, or Rough-lemon rootstock. One or two specific elements, which varied from species to species, were found in low concentration in one or the other of the scion-rootstock combinations.

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THE IRON–MANGANESE RELATION IN PLANT METABOLISM[1]

I. I. Somers and J. W. Shive
(WITH SIX FIGURES)

The results of many experiments have clearly demonstrated the prime importance of trace elements in the nutrition of plants. The small amounts of these substances required indicate that they have a unique function. Two of the trace elements, iron and manganese, have been given broad consideration because of their association, in one way or another, with the important plant disease or diseases commonly known as chlorosis. The problem of chlorosis in plants has been investigated by many plant scientists because of its urgent agricultural significance.

The literature is replete with reports of field tests (**2, 4, 14, 19, 20**) in which iron and manganese have been added to the soil to bring about recovery of plants showing symptoms of the common chlorotic disorders known as "yellows," "grey speck of grains," "marsh spot of peas," and many other similar diseases. The problem is not a simple one, and frequently the results of analytical data are conflicting and tend to confuse the problem rather than clarify it. For example, several experiments have been reported, (**5, 6, 11, 13, 14**) in which chlorotic plants, when analyzed, were found to have a higher iron content than normal green plants. This suggests that much of the iron present in chlorotic plants is in an insoluble form and is not effective in the metabolic processes.

Investigation of the problem of iron availability (**14, 20**) has led to general acceptance of the theory that it is the soluble form in the plant which plays the important rôle in iron metabolism. It is evident that this element, by being precipitated within the tissues, may become inactivated and therefore unavailable to metabolic processes of the plant. When this is considered in connection with the work of Johnson (**8**), which shows that ferrous iron is soluble at relatively high pH values whereas ferric iron is precipitated at a relatively low pH value, it is logical to assume that the excessive proportion of inactive iron found in chlorotic plants has been oxidized from the ferrous to the ferric form, which is then precipitated. Just how oxidation may occur is not fully understood, nor is it clear by what mechanism the reduction of ferric iron is suppressed in the plant. The work of Kelley (**10**) and Johnson (**9**) is significant since it has shown that certain Hawaiian Island soils contain an abundance of soluble iron, react acid to litmus, and yet pineapple plants grown on them acquired a chlorosis which could be cured by spraying the plants with iron solutions. These soils were found to contain also

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large amounts of manganese, and it was noted that the higher the manganese content of the soil the more severe were the symptoms of chlorosis. This led to the suggestion that manganese might act as an oxidizing agent in rendering iron unavailable to the plant. The work of HOPKINS (**7**) also suggests that manganese may be physiologically effective in determining the state of oxidation of iron within the plant.

It is evident from the results of the various experiments that the elements iron and manganese are functionally interrelated in some way; very little attention has been given, however, to a study of such interrelationships. The work of TOTTINGHAM (**18**) is probably the most quantitatively significant in this respect. He reported that the best growth of wheat could be obtained when the ratios of iron to manganese in the substrate were approximately 1 to 2.5, which is strong evidence of an interrelationship.

The work here reported was undertaken for the purpose of making a systematic study of possible interrelations between iron and manganese in their effects upon the metabolic processes of plants, with the hope of arriving at some definite understanding of these interrelations.

Methods

CULTURAL METHODS

Soybeans were chosen as the indicator plants in this investigation because preliminary experiments showed them to possess a relatively high manganese requirement for normal growth and development.

The general culture methods were similar to those used and described by GILBERT (**3**), which are a modification of the continuous flow method as outlined by SHIVE and ROBBINS (**17**). The modified method made use of glass tubes about 4 to 8 cm. long, and of 0.5 to 1 cm. bore, depending on the plant used, projecting through a rubber stopper, which was fitted into the top of the jar, for holding the plants. The tubes projected about 2 cm. below the solution surface. This isolated the free space over the solution from the external environment, leaving the solution and roots completely enclosed and in contact only with glass; this eliminated the danger of trace element contamination by dust particles, metal caps, or cork stoppers.

The soybeans were germinated between moist blotter papers. Immediately after germination the seed coats were removed to eliminate as much manganese as possible since MCHARGUE (**12**) found a great accumulation of this element in seed coats. The seeds were then put on a paraffined cloth net stretched tightly over a pan of solution and placed in the dark to allow the hypocotyls to elongate. As soon as the hypocotyls were about 5 to 6 cm. long, the plants, roots first, were pushed through the glass tubes into the culture jars nearly filled with solution.

The following basic solution was used for all experiments:

	Mol concentration	Milliliters of M/2 stock solution added per liter	Total p.p.m.
KH_2PO_4	0.00058	1.16	K 86.2
			Ca 180.0
K_2SO_4	0.00082	1.63	P 18.0
			S 94.8
$Ca(NO_3)_2$	0.00450	9.00	Mg 52.3
$MgSO_4$	0.0023	4.60	N 126.0

A boron concentration of 0.5 p.p.m. was maintained in this basic solution, and iron and manganese were added as described in connection with each experiment.

The pH of each culture was maintained between 4.6 and 4.8. This was done in order to hold the iron as completely as possible in a soluble, readily available form in the culture solution. Preliminary experimental work showed that injury due to low pH did not occur above pH 3.5, if the solution contained no ammonium nitrogen. In order to keep the pH level as constant as possible to prevent the loss of iron by precipitation three control measures were employed: first, the solutions were completely changed every other day; second, new solution was added by the continuous flow method at the rate of 1.5 liters per day; third, the pH of the drain solutions was checked daily.

All salts used were biologically tested for iron and manganese impurities. The test was made by growing plants in nutrient solutions of the salts to determine whether manganese deficiency and iron deficiency symptoms could be produced. In the biological test solution for manganese, iron was added at the rate of 0.5 p.p.m., and in the biological test solution for iron, manganese was added at the rate of 0.25 p.p.m.

At the time of harvest the plants were taken from the culture jars and thoroughly washed in distilled water to free them from all traces of dust. All harvesting was done between 10:30 A.M. and 2:00 P.M. The tops were separated from the roots by breaking them off (by hand) at the lower end of the glass supporting tubes. The roots were divided into two similar portions, shaken, rapidly blotted with absorbent paper to remove excess water, and each portion was weighed. The tops were likewise separated into two similar portions, each of which was weighed. The leaf blades and buds were removed from the stems and weighed. After the weights were taken one portion of each of the tissues was rapidly frozen while the duplicate lot was dried. Freezing was accomplished with an ice salt mixture. The frozen samples were stored in a refrigerator at a temperature below freezing, until analyzed.

As a means of studying pathological symptoms, plants were observed very closely at all times, and at harvest the approximate percentage of the plants severely affected was recorded.

The active fractions of iron and manganese in the plants are here as-

sumed to be the proportions of these elements which are in solution within the cells. This fraction was extracted as completely as possible under high pressure. The juices of the tissues thus obtained were filtered and analyzed separately from the press-cake. Manganese and iron in the filtrate are here assumed to be the active, soluble fraction, although this fraction may not represent the absolute total of all the active ions of these elements in the tissues. The proportion of the elements remaining in the press-cake is regarded as the inactive or unavailable fraction. It is to be emphasized also that this method may not be an absolute quantitative differentiation between the soluble and insoluble fractions of these elements in the tissues, since a water extraction of the press-cake sometimes reveals traces of the elements which cannot be extracted by pressure alone.

General analytical methods

The frozen tissue was thawed by placing it on a small piece of muslin in the Carver press pan. It was then wrapped in muslin and placed in the pressure chamber at 1600 pounds per square inch applied for 2.5 minutes. The juice was filtered into a 50-ml. volumetric flask through a no. 40 Whatman filter paper. The press-cake was taken out of the muslin, placed in a drying basket, covered, and dried in an oven at 65° C. for 48 hours. The muslin was washed three times by wetting it with distilled water and wringing the water out on the filter paper. All the press equipment was washed with distilled water and the wash liquid poured on the filter. The filter paper was ashed and added to the cake sample. The filtrate sample was made to volume with water and thoroughly mixed. The press-cake was weighed after drying and was ground to a fine powder in a Wiley mill. Preliminary tests showed the iron content of identical samples to be the same whether ground with mortar and pestle or in the Wiley mill.

Iron determination.—Aliquots of the juice or of the ground tissue were placed into micro-Kjeldahl flasks and wet-digested very slowly (just at boiling point at first and later with slow simmering) with 1 ml. of concentrated sulphuric acid and 0.3 ml. of perchloric acid. The excess water was removed from the filtrate samples by placing the Kjeldahl flask containing the sample and 4 drops of concentrated sulphuric acid in a boiling salt solution until the water had evaporated, leaving the acid and organic matter in the flask. To this was then added the remainder of the sulphuric and perchloric acids, and digestion was carried out on a micro-Kjeldahl digestion rack. After digestion, the samples were transferred to 50-ml. volumetric flasks and the iron was determined according to the Saywell and Cunningham orthophenanthroline method (**16**), using a modified single-celled, photoelectric colorimeter. The determinations were carried out in duplicate or triplicate.

MANGANESE DETERMINATION.—Aliquots of both juice and ground tissue were taken and wet-digested very slowly, as outlined for the iron determinations, in graduated 25-ml. Kjeldahl flasks. After digestion, 10 ml. of water and 0.2 gm. of potassium periodate were added. The flasks were then placed in a boiling water bath, or in an oven at 95° C., shaken every two minutes until the color of purple-red permanganate appeared, and then shaken thoroughly every five minutes for at least two more hours. Best results were obtained when the samples were left in the oven overnight, and this was done for all determinations here reported. After the permanganate color had been developed, the samples were made to a volume of 25 ml. with boiled distilled water and the amounts determined by getting the reading on a photoelectric colorimeter and interpolating this reading on a curve made by plotting the readings of a series of standards. This method was checked by running pure manganese sulphate through the complete method. An accuracy of $\pm$ 3 per cent. was obtained with larger amounts of manganese, and $\pm$ 6 per cent. on the smaller amounts. This method was adapted from the A.O.A.C. method (**1**) after a consideration of the wet-digestion method of PALFREY and co-workers (**15**). With this method it is important that the digestion be carried to completion to insure that all hydrochloric acid which may result from the breakdown of the perchloric acid has been driven off. This is indicated by the sequence of clearing and coloring of the digestion solution. As the digestion continues, the solution clears and as this is continued further the solution colors again and digestion must continue until the solution has cleared the second time. When this is done, good reproducible results are obtained.

Iron and manganese in the growth of soybeans

PLAN OF EXPERIMENTS

These experiments were designed primarily to study responses of the soybean plants and also to study the influence of the two elements here considered on the presence and form of the other within the plant.

Eighteen cultures, each containing six plants, were divided into three series of six cultures each. Each series was grown at a different level of iron supplied as ferrous sulphate. The first was grown at a concentration of 0.005, the second at 0.5, and the third at 3.0 p.p.m. Manganese as manganese sulphate was added at different concentrations in each of the six cultures at the three iron levels. The manganese concentrations at each iron level were as follows: 0.00, 0.002, 0.01, 0.25, 2.0, and 5.0 p.p.m. To facilitate designation of the particular treatments the cultures are numbered consecutively from 1 to 18 in table I. Series 1 includes cultures 1 to 6; series 2, cultures 7 to 12; and series 3, cultures 13 to 18, inclusive. The experiments were run in duplicate. The cultures at each treatment will be designated collectively

TABLE I

CULTURE (BLOCK) NUMBERS, PATHOLOGICAL SYMPTOMS, AND IRON-MANGANESE CONCENTRATIONS IN SERIES 1, 2, AND 3

CULTURE BLOCK NUMBER	CONCENTRATIONS IN SUBSTRATE		SYMPTOMS AT HARVEST	SYMPTOMS APPARENT IN DAYS
	Fe	Mn		
	p.p.m.	*p.p.m.*		
Series I				
1	0.005	0.000	None	....
2	0.005	0.002	None	....
3	0.005	0.010	Slight	11
4	0.005	0.250	Medium	4
5	0.005	2.00	Severe	2
6	0.005	5.00	Very severe	2
Series II				
7	0.500	0.000	Very severe	6
8	0.50	0.002	Severe	7
9	0.50	0.010	Medium	9
10	0.50	0.250	None	....
11	0.50	2.00	Slight	7
12	0.50	5.00	Medium	4
Series III				
13	3.00	0.000	Very severe	5
14	3.00	0.002	Very severe	6
15	3.00	0.010	Medium	7
16	3.00	0.250	None	....
17	3.00	2.00	None	....
18	3.00	5.00	Slight	7

as a block of cultures. Treatments were started twelve days after germination and the plants were harvested 18 days later.

PATHOLOGICAL SYMPTOMS

A graphic representation of the development of pathological symptoms of the plants at each of the three iron levels is presented in the upper half of figure 1.

Two days after treatments were started the first symptoms of manganese toxicity appeared in the plants of the high manganese cultures, blocks 5 and 6 (fig. 1, shaded area on the right). The symptoms were identical with those of iron deficiency. Three days later the first manganese deficiency symptoms appeared in the low manganese cultures, block 13, and one day later in the plants of block 7. The symptoms observed in the plants of blocks 7 and 13 (fig. 1, shaded area on the left) were distinctly different from those resulting in the plants of blocks 5 and 6, but were identical with those usually regarded as iron toxicity symptoms.

To study and designate these differences in the pathological symptoms a very careful inspection of each culture was made and the composite symptoms related to each treatment were listed.

Symptoms of manganese deficiency corresponding to iron toxicity

The characteristic symptoms were observed in the plants from the culture of blocks 7, 8, 9, 13, 14, and 15.

Early symptoms.—The first indication of a disorder was a gradual fading of the green color of the lower leaves. As the severity increased the fading developed into a yellowing between the veins at the basal ends of

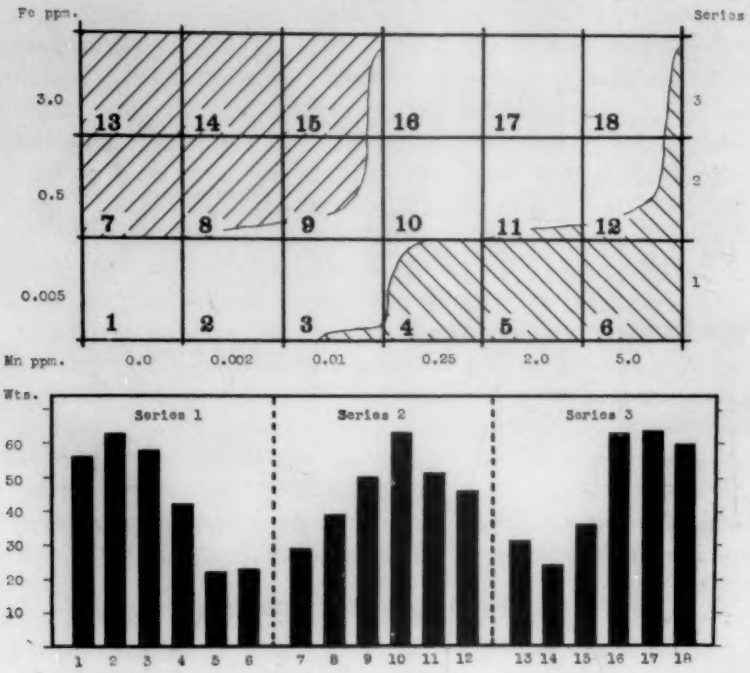

Fig. 1. Upper half represents the cultures of the three series in which the shaded portion of each block indicates the approximate percentage of the plants showing pathological symptoms. The shaded area in the upper left indicates iron toxicity or manganese deficiency, and the shaded area in the lower right indicates iron deficiency or manganese toxicity. The unshaded area represents good plants free from pathological symptoms. Lower half of figure represents average green weights of tops of plants in each block of cultures.

the leaves; as the symptoms progressed further the growth of the plants was retarded.

Late symptoms.—As the disorder became increasingly severe there was a fading of the green color between the veins of the upper leaves which became spotted with very small, brown, necrotic areas (C, fig. 2). In the most severe cases the new leaves opened from the buds in a faded condition with the necrotic areas already formed, and in many cases these leaves failed to

develop, abscissed, and dropped off. The stem growing points of severely affected plants died. The roots of the affected plants were smaller than those of normal plants but otherwise showed no visual symptoms.

Symptoms of manganese toxicity corresponding to iron deficiency

Characteristic visual symptoms of manganese toxicity appeared in the plants of blocks 4, 5, 6, 11, 12, and 18 (fig. 1). The first indication of any

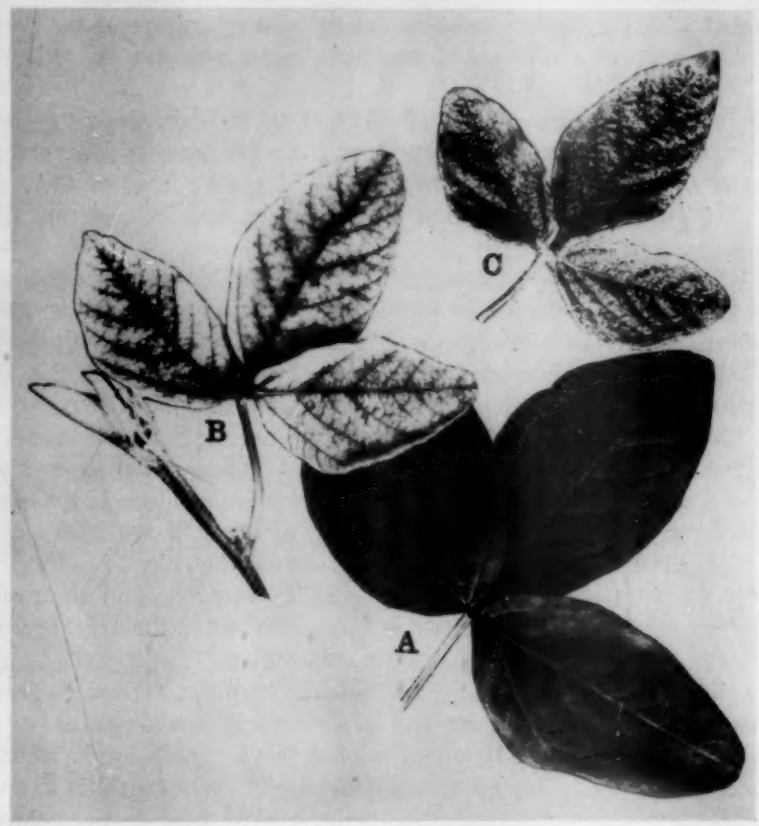

Fig. 2. A, leaf of a normal plant from block 10, fig. 1. B, leaf of a plant from block 6, showing symptoms of iron deficiency (manganese toxicity). C, leaf of a plant from block 13, showing iron toxicity (manganese deficiency).

disorder was the appearance of a slight discoloration or tanning of the roots. This was followed by a yellowing and slight curling of the upper leaves (fig. 2, B).

The roots became progressively browner in color, and the yellowing of the upper leaves continued until, finally, the newer leaves were almost paper-

white (fig. 2, B). This was accompanied by a downward curling of the leaves and a darkening of the midribs in some of the leaves. This darkening appeared somewhat purple in color but was not a normal anthocyanin pigmentation since it was accompanied by a breakdown of the tissue. Numerous large necrotic areas appeared on the most chlorotic leaves and the necrosis was usually accompanied by death of the stem growing points. The roots of these plants stopped growing very soon after the first symptoms appeared, and on examination under the microscope the brown color appeared to be associated with the darkening of the nuclei and cytoplasm of the cortical cells, along with the occurrence of numerous brown masses in the cells which could not be identified.

The symptoms of manganese deficiency (iron toxicity) are very distinct in nature from those of manganese toxicity (iron deficiency); therefore, they can be used as criteria in the interpretation of plant responses and to distinguish between iron or manganese deficiencies and iron or manganese toxicities.

It is important to note from the location of the blocks that the healthy plants free from pathological symptoms occurred in cultures with extreme variations in both the manganese and iron concentrations of the substrate. At the three different iron levels the blocks of cultures in which the plants show no pathological symptoms and which produced highest average yields are 2, 10, and 17. This is of special interest since the manganese and iron concentrations of the solutions in block 10 are 125 and 100 times higher, respectively, than the concentrations of the solutions of block 2; the manganese and iron concentrations in the solution of block 17 are 1000 and 600 times higher, respectively, than those in the solution of block 2. Each produced the highest yield in its respective series, however, and the average yields from these three blocks of cultures are approximately equal, as indicated in the lower portion of the diagram of figure 1.

Excellent plants, free from pathological symptoms related to iron or manganese deficiency or toxicity, can be grown within wide ranges of concentrations of both iron and manganese, but this is possible only when the concentration of both elements in the substrate of the cultures is varied at the same time, so that the approximation of a fixed ratio between the concentrations of the two elements is maintained. This emphasizes the balance between these elements in a given substrate of growth, and further demonstrates that the absolute concentrations of these elements supplied in the nutrient substrate are not particularly important; it is the concentration of the one in relation to the concentration of the other element present which is effective in maintaining an adequate growth status. To further emphasize this point it may be observed from table I and figure 2–B that a severe pathological condition is manifested by the plants of block 6 in which the

ratio of iron to manganese is completely out of balance for effective growth. It was possible to correct this chlorotic condition in two ways: first, by reducing the manganese concentration to restore the effective balance between iron and manganese in the substrate; and second, by increasing the iron concentration to restore the effective iron-manganese ratio. It was also possible to correct the pathological symptoms exhibited by the plants in block 13 in a similar manner: first, by decreasing the iron concentration without change in the manganese concentration; and second, by increasing the manganese concentration without change in the iron concentration so as to restore the effective iron-manganese balance.

TABLE II

AVERAGE GREEN AND DRY WEIGHT YIELDS AND RATIOS OF IRON TO MANGANESE IN SUBSTRATE

CULTURE BLOCK NUMBER	CONCENTRATION IN SUBSTRATE		AVERAGE GREEN WEIGHT	AVERAGE DRY WEIGHT	RATIO OF Fe/Mn IN SUBSTRATE
	Fe	Mn			
	p.p.m.	*p.p.m.*	*gm.*	*gm.*	
Series I					
1	0.005	0.00	56.0	3.47	0.0
2	0.005	0.002	63.0	3.78	2.50
3	0.005	0.010	58.2	3.62	0.50
4	0.005	0.250	42.5	2.86	0.02
5	0.005	2.00	21.7	1.50	0.0025
6	0.005	5.00	22.5	1.57	0.0010
Series II					
7	0.50	0.00	29.0	1.51	0.0
8	0.50	0.002	39.3	2.22	250.0
9	0.50	0.010	50.1	3.01	50.0
10	0.50	0.250	63.2	3.78	2.0
11	0.50	2.00	51.4	3.16	0.25
12	0.50	5.00	46.2	3.02	0.10
Series III					
13	3.00	0.00	30.7	1.37	0.0
14	3.00	0.002	23.5	1.39	1500.0
15	3.00	0.010	34.7	1.90	300.0
16	3.00	0.250	62.5	3.78	12.0
17	3.00	2.00	64.1	4.05	1.8
18	3.00	5.00	60.4	3.70	0.6

It becomes quite apparent, from the above discussion, that in any interpretation of either the observational or the analytical data provided by this investigation, it would be futile to consider the responses of the plant toward one of these elements separately from its responses to the other; the effect of one of these elements upon the plant, other things being equal, is determined by the proportionate presence of the other, both in the substrate of growth and in the plant tissues. This will become clear from the following discussion. Symptoms of manganese deficiency in the plants here employed

must be regarded as identical with the disease known as iron toxicity, and manganese toxicity in the soybean is the same disease as that resulting from iron deficiency.

The green and dry weight values of the cultures are given in table II and are shown diagrammatically in the lower half of figure 1. These values further emphasize the interrelationship pointed out above between the elements, iron and manganese, in their effects upon the plant. They demonstrate that the green and dry weight production of the plant, other things being equal, is determined by the ratios of these elements in the substrate. For example, figure 1 shows that a shift from low iron (0.005 p.p.m.) to high iron (3.0 p.p.m.) concentrations of the substrate causes a corresponding shift in the concentration of manganese necessary to produce optimum growth. In other words, when iron was low in the substrate maximum growth was obtained when the manganese was at the concentration of 0.002 p.p.m. When the iron in the substrate was increased to 0.5 p.p.m., however, maximum growth was obtained when the manganese concentration was 0.25 p.p.m. Likewise, when iron was present in the substrate at the concentration of 3.0 p.p.m., optimum growth was obtained when the manganese was at the concentration of 2.0 p.p.m. Approximately the same yield was produced at the three different levels of iron concentrations (low, medium, and high) but if the iron concentrations do not balance the manganese concentrations in the ratio of approximately two to one, yield production is greatly diminished and pathological symptoms appear as indicated in the diagram, figure 1.

It is thus evident from the green and dry weight values as well as from the deficiency-toxicity symptom studies that there is very definitely an interrelation between iron and manganese; that this relationship is centered around the maintenance of an appropriate balance between these elements supplied to the substrate; and that the approximate value of the ratio of iron to manganese which will produce good growth of soybeans, under the experimental conditions here employed, was found to be about 2. Within a narrow range on each side of this ratio value, good plants, free from pathological symptoms, were grown.

Chemical analytical results

The results of the analyses of the plants for iron and manganese are given in tables III and IV. Table III gives the values for leaf tissues, and table IV the corresponding values for the roots.

In general, the trends indicated by quantities of iron and manganese in the roots roughly parallel those in the leaves, but the iron analyses for roots invariably show higher values than do those for leaves. This is particularly true of the analyses of roots grown at the high iron level, which shows excessively high values. It is suspected that these high iron values for

roots may be due to precipitated iron which adheres to the root surfaces and which cannot be removed by washing. For this reason the results of root analyses will not be emphasized in the consideration of analytical data.

The total iron and manganese of the plants increased with increase in the concentration of these elements in the substrate, thus indicating that the

TABLE III

SOLUBLE, INSOLUBLE, AND TOTAL IRON AND MANGANESE IN TERMS OF MILLIGRAMS PER GRAM OF DRY TISSUE OF SOYBEAN LEAVES AND RATIOS OF SOLUBLE IRON TO SOLUBLE MANGANESE

CULTURE (BLOCK) NUMBER	MANGANESE			IRON			RATIO SOL. Fe / SOL. Mn
	SOLUBLE	INSOLUBLE	TOTAL	SOLUBLE	INSOLUBLE	TOTAL	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Series I							
1	0.0155	0.0149	0.0304	0.0410	0.2095	0.2505	2.65
2	0.0169	0.0152	0.0321	0.0400	0.1775	0.2175	2.37
3	0.0173	0.0344	0.0517	0.0355	0.1687	0.2042	2.05
4	0.0363	0.2580	0.2943	0.0362	0.1773	0.2135	1.00
5	0.0836	0.9990	1.0826	0.0672	0.1558	0.2230	0.80
6	0.0518	0.7890	0.8408	0.0452	0.1727	0.2159	0.83
Series II							
7	0.0096	0.0018	0.0114	0.0643	0.2760	0.2403	5.66
8	0.0092	0.0019	0.0111	0.0500	0.2460	0.2960	5.44
9	0.0095	0.0026	0.0121	0.0424	0.2482	0.2906	4.47
10	0.0200	0.0331	0.0531	0.0380	0.2585	0.2974	1.95
11	0.0455	0.1734	0.2189	0.0329	0.2560	0.2889	0.72
12	0.0515	0.3600	0.4115	0.0301	0.2260	0.2561	0.58
Series III							
13	0.0107	0.0017	0.0124	0.1050	0.5180	0.6220	9.82
14	0.0108	.....	.....	0.1063	.....	.....	9.85
15	0.0104	0.0023	0.0127	0.1027	0.2880	0.4907	9.88
16	0.0190	0.0136	0.0326	0.0480	0.2960	0.3440	2.48
17	0.0286	0.1024	0.1310	0.0428	0.2800	0.3228	1.50
18	0.0424	0.2370	0.2794	0.0322	0.2325	0.2657	0.76

absorption rates of these elements are determined largely by their concentrations in the substrate.

Since the amounts of the elements absorbed by the plants are related to their concentrations in the substrate, and since their concentrations in the substrate must be maintained within a narrow range of values of the iron-manganese ratio for effective growth, a study was made of the soluble and insoluble fractions of these elements within the tissue to determine whether a relationship between iron and manganese within the tissues, similar to that in the substrate, is required for the development of a healthy plant free from pathological symptoms. Ratios between total iron and total manganese within tissues showed no definite relation to any particular deficiency-toxicity condition or to normal plant growth. The ratios of the soluble fractions, however, especially those for the leaves, did show a narrow

range of values which was definitely associated with good growth and development while all the values outside of this limited range were related to metabolic disturbances which manifest themselves in visible deficiency-toxicity symptoms.

The ratios of soluble iron to soluble manganese in the tissues of tops are presented in table III. These ratios have been plotted against manganese concentrations of the substrate at each of the three iron levels in the graphs of figure 3. The values representing the ratios of (active) soluble iron to

TABLE IV

SOLUBLE, INSOLUBLE, AND TOTAL IRON AND MANGANESE IN TERMS OF MILLIGRAMS PER GRAM OF DRY TISSUE OF SOYBEAN ROOTS, AND RATIOS OF SOLUBLE IRON TO SOLUBLE MANGANESE

CULTURE (BLOCK) NUMBER	MANGANESE			IRON			RATIO Sol. Fe / Sol. Mn
	SOLUBLE	INSOLUBLE	TOTAL	SOLUBLE	INSOLUBLE	TOTAL	
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Series I							
1	0.0243	0.0000	0.0243	0.1223	0.3490	0.4613	5.04
2	0.0329	0.0063	0.0392	0.1105	0.3518	0.4623	3.36
3	0.0368	0.0234	0.0602	0.1003	0.3508	0.4511	2.73
4	0.0725	0.7180	0.7905	0.1042	0.3875	0.4917	1.44
5	0.1496	1.532	1.681	0.2023	0.5470	0.7493	1.35
6	0.1358	1.258	1.394	0.1748	0.5706	0.6454	1.29
Series II							
7	0.0173	0.0000	0.0173	0.2728	15.80	16.07	15.8
8	0.0189	0.0000	0.0189	0.2745	15.41	15.68	14.5
9	0.0188	0.0000	0.0188	0.2332	14.28	14.51	12.4
10	0.0431	0.0494	0.0925	0.0964	13.12	14.08	2.24
11	0.0676	0.4830	0.5506	0.0855	12.56	13.44	1.26
12	0.0686	0.5610	0.6296	0.0804	11.93	12.73	1.17
Series III							
13	0.0175	0.0000	0.0175	0.3050	35.85	36.15	17.4
14	0.0181	0.0000	0.0181	0.2737	35.98	36.25	15.1
15	0.0184	0.0000	0.0184	0.2458	35.60	35.84	13.3
16	0.0253	0.0000	0.0253	0.1048	35.58	35.69	4.15
17	0.0368	0.0220	0.0588	0.0922	35.01	35.10	2.51
18	0.0488	0.0616	0.1104	0.0862	34.30	35.39	1.77

(active) soluble manganese which are definitely associated with normal healthy plants for each of the three iron concentration series all lie within the narrow unshaded area extending horizontally across the diagram of figure 3. The range of these ratio values extends from 1.5 to 2.5. From this, it is apparent that within certain limits not attained in these studies, total quantities of these elements in the plants, as in the substrate, are comparatively unimportant; but the quantity relation which one bears to the other is significant. Values above this effective range are definitely related to iron toxicity or manganese deficiency in the plants, and those below this range are associated with visible iron deficiency or manganese toxicity symptoms.

It is also significant that the iron-manganese ratios in the substrate and in the tissues, which are associated with good growth and development of plants free from pathological symptoms, show approximately the same values. The range of effective values, however, may be somewhat wider in the plant tissues than in the substrate.

As has already been mentioned the concentration of both iron and manganese in the substrate determines the total quantities of these elements within

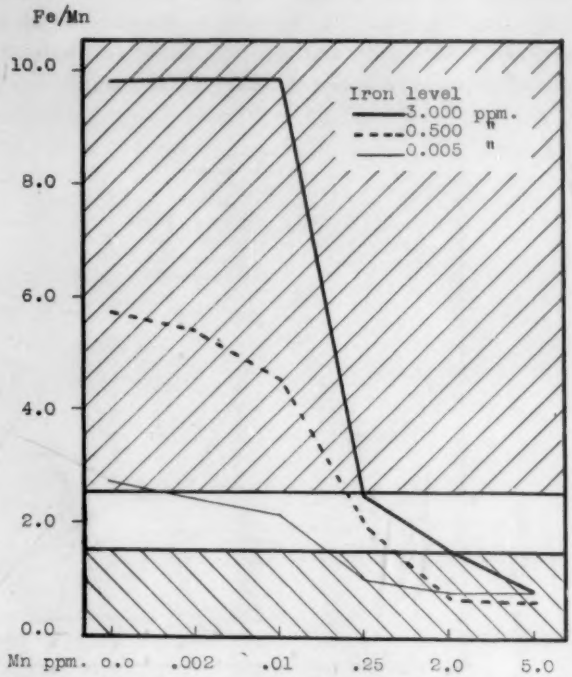

FIG. 3. Ratios of soluable iron to soluble manganese in soybean tops plotted against manganese concentrations in the substrate at three iron levels.

the plant. This is clearly indicated by the data of table III, and by the graphs of figure 4 which represent the average values of total iron in the leaves of the plants for each of the three series plotted against the manganese concentrations of the substrate. The graphs show that iron accumulation in the leaves at each of the three iron levels is always higher at low than at high manganese concentration in the substrate. This relation, however, is not particularly significant in the series at the low iron level, but becomes progressively more pronounced at the higher iron levels. In the series at the highest iron level the average total iron in the leaves at the lowest manganese concentration is more than double that at the highest manganese

concentration. Furthermore, at high manganese concentrations the accumulation of iron by plants grown at a high iron level in the substrate is no greater than it is at a much lower iron level when the manganese concentration is correspondingly low in the substrate. It is thus apparent that iron and manganese show reciprocal relations in the accumulation of these elements in the tissues and in the effects upon the plant resulting from their functional activities in the metabolic processes. The importance of these reciprocal relations, however, can be set forth much more clearly by a consideration of the data representing the soluble fractions of these elements in the tissues involved. It is here assumed, of course, that these elements can be functionally effective only in the soluble (active) state.

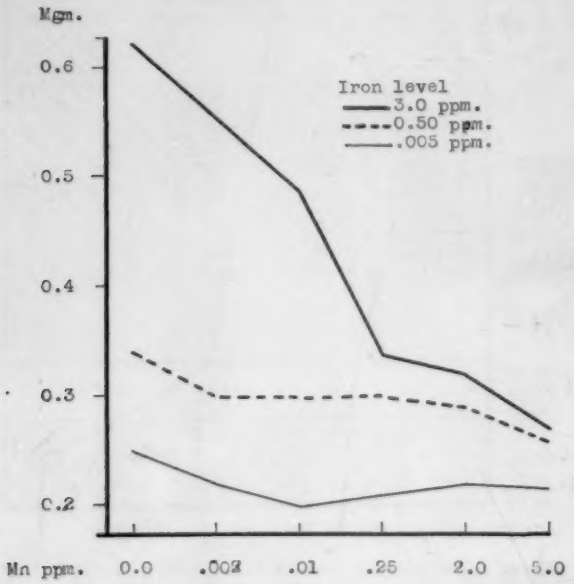

FIG. 4. Total iron in tops of soybean plants, in terms of milligrams per gram of dry tissue, at three iron levels plotted against manganese concentrations in the substrate.

The analytical data of series 2 are presented in table III, and are graphically represented in figure 5. Soluble iron and soluble manganese in terms of milligrams per gram of dry leaf tissue are plotted against manganese concentrations of the substrate. The corresponding data of the cultures of series 1 and series 3, conducted at the very low and the high iron levels, respectively, are not graphically presented since the data of series 2 are representative of all three series; the general principles illustrated by the graphs of figure 5, however, are slightly less pronounced when referred to series 1, but much more emphatic when referred to series 3.

The graphs show clearly that low manganese in the substrate corresponds to low soluble manganese and high soluble iron in the tissues. Excessively high soluble iron in the tissues invariably results in the specific symptoms of iron toxicity. On the other hand, high manganese concentrations in the substrate correspond to high soluble manganese and low soluble iron in the tissues, invariably resulting in the typical chlorosis due to iron deficiency. Thus at any given iron level, within the range of concentrations here employed, soluble iron in the plant may be toxic, effective for good growth and development, or deficient depending upon the presence, simultaneously, of the relative proportion of soluble manganese. As already indicated, if the ratio of soluble iron to soluble manganese in the plant is outside of the effective range extending approximately from 1.5 to 2.5, pathological symptoms

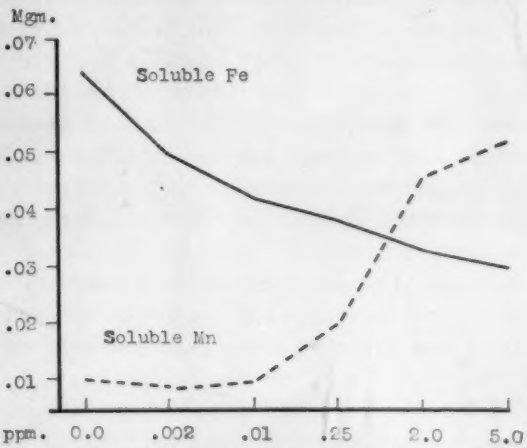

FIG. 5. Soluble iron and soluble manganese in the tops of the plants of series 2 (Fe, 0.5 p.p.m.) plotted against manganese concentrations in the substrate.

appear in the soybeans here employed as test plants. It is not to be expected, however, that the same range of the iron-manganese ratio values which has proved effective for good growth and development of the soybean plant would be effective for all species with reference to either the nutrient substrate or the metabolically active plant.

The experimental evidence here presented clearly demonstrates that the two elements, iron and manganese, are intimately interdependent in their effects upon the plant and that the nature of the effects of the one is determined by the proportionate presence of the other. Such relationships between these two elements, relative to their influence in living systems have been suggested by many investigators, and the suggestions have usually been prompted by the fact that symptoms of chlorosis and other characteristic effects, resulting from deficiency or excess of one of the elements are either

identical with or are reciprocally related to the effects produced by the other.

The theoretical explanation of the important rôle which iron plays in the plant processes in which manganese functions actively, centers around two important facts: first, that metabolically reactive iron functions primarily in the reduced state, that is, in the ferrous condition; and second, that the oxidizing potential of manganese is higher than that of iron.

When iron is present in a nutrient substrate and is absorbed by the roots of the plant it is retained in the active, ferrous condition under the influence of the strong reducing systems of the living cells, unless it falls under a sufficiently energetic counter-reactant and is oxidized to the ferric state. If such a counter-reactant is absent or is present only in deficient quantity, a low concentration of iron may become a powerful toxic agent and produce pathological symptoms, as clearly shown by the experimental evidence here reported. If, however, a counter-reactant in the form of a strong oxidizing agent, such as manganese, with a high oxidizing potential, is present in adequate concentration, the active iron may be oxidized to the ferric state, in which condition it is precipitated in the form of some ferric-phosphorus organic complexes and rendered biologically inactive. The experimental data show that manganese does exercise such a restraining influence upon active iron in the plant system. Furthermore, manganese possesses the chemical characteristics necessary for the maintenance of a theoretical equilibrium system in the plant in accordance with some scheme such as is proposed in figure 6, and in some such manner as above described, but the sequence of events and the details of the exact processes are complex and not at all understood.

Assuming that such a dynamic relationship exists in the living plant system, and there is considerable experimental evidence to substantiate that it does exist, it then follows that as long as a supply of iron and manganese is available in the nutrient substrate, a dynamic equilibrium system involving both oxidized and reduced ions of both iron and manganese must be maintained in the plant. It is obvious, however, that complete oxidation or complete reduction of iron or manganese cannot be attained in such a balanced system as long as an adequate supply of the two elements is available in the nutrient substrate and absorbed by the plant, but the oxidation of ferrous ions to ferric ions and then the precipitation of iron in the form of ferric organic complexes is determined by the relative quantity of manganic ions present in the system. Thus, when the relative proportion of manganic ions is excessive, the biologically active ferrous ions are maintained at a proportionately low level by the strong oxidizing effect of the manganic ions; but when the relative proportion of manganic ions is deficient, the active iron capable of functioning in the metabolic processes is maintained at a proportionately high level under the influence of the strong reducing sys-

tems in the living cells. When these active ferrous ions become excessive in the living tissues through a shift in the dynamic equilibrium in their direction because of a deficient supply of manganese in the nutrient substrate, the plant will manifest the excess of active ferrous ions by the development of pathological symptoms. If, on the other hand, the equilibrium is shifted in the opposite direction, as it is with excessive manganese in the substrate, so that a deficiency of active iron results, the plant will manifest this also by the development of pathological symptoms. In the one case the plants show symptoms of iron toxicity, identical with those of manganese deficiency; in the other case, they show symptoms of iron deficiency, identical with those of manganese toxicity.

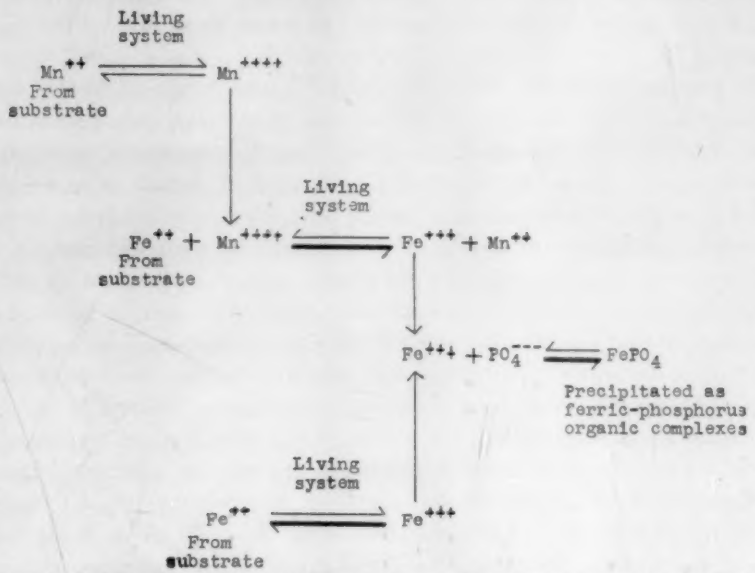

FIG. 6. Scheme illustrating a possible theoretical equilibrium system in which iron and manganese are functionally related.

The equilibrium between ferrous and ferric ions should determine the amount of insoluble iron in the plant, since ferric ions are much more likely to be precipitated than are the ferrous ions. Phosphorus was investigated as a possible factor in this precipitation and it was shown by experimentation that an increase of the phosphorus content of the plants brings about an increase in the percentage of the insoluble iron within the leaf tissues. It is thus logical to assume that this precipitated iron is some form of a ferric-phosphorus-organic complex as indicated in the reaction diagram (fig. 6). The results of this experimentation also demonstrate that since ferric ions are rapidly precipitated in the plant, the soluble fraction must

be largely in the reduced form. This also indicates an equilibrium within the plant between ferrous and ferric ions, which in turn are in equilibrium with an insoluble iron complex.

The experimental results show that an increase in manganese brings about a decrease in the soluble iron and an increase in the percentage of insoluble iron in the plant. This indicates that manganese, with its high oxidizing potential, is responsible for the decrease of soluble iron by oxidation of the ferrous ions to ferric ions, which ultimately results in the precipitation of iron. The fact that KELLEY (10) found crystals of manganese dioxide in the tissues of plants that showed manganese toxicity also fits into this scheme. Furthermore, since the oxidation potential of manganese is higher than that of iron it may exert a preventive action against the reduction of iron by the reducing systems of the plant, as shown by the work of HOPKINS (7).

To put the oxidation-reduction hypothesis here proposed to still further test, a series of cultures was carried out using seedling corn plants for the tests. In these cultures cobalt was substituted for manganese in equivalent concentrations. Since the oxidation potential of cobalt is considerably higher than that of manganese, it should have a stronger tendency to reduce the metabolic efficiency of iron than does manganese, provided that the effect is related to the dynamic equilibrium system involving the metallic ions and the oxidation-reduction systems of the cells. The results showed very emphatically that cobalt was more effective than manganese in reducing the soluble iron content of the plants, and this was further emphasized by the greater speed with which iron deficiency symptoms appeared in the plants when cobalt was substituted for manganese at the higher concentrations. The pathological symptoms developed by the plants were identical with those developed when manganese was in excess. It is, of course, not to be assumed that manganese could be replaced effectively by cobalt throughout the life cycle of the plant. No tests were made, however, to determine this point, nor has the essentiality of cobalt for growth and development yet been established.

Summary

1. Experiments were carried out with soybeans in solution culture at three different iron levels at each of which the manganese concentrations were varied through a relatively wide range. Studies were made of the interrelations between iron and manganese in their effects upon the metabolic processes of the plants. Toxicity and deficiency quantities of the two elements in the substrate and in the plants were investigated and their relations to the resulting pathological symptoms were studied.

2. Pathological symptoms produced with excessive iron are identical with those produced when manganese is deficient, and the symptoms produced

with excessive manganese are identical with those produced when iron is deficient. That is, symptoms of iron toxicity correspond to those of manganese deficiency, and symptoms of manganese toxicity correspond to those of iron deficiency.

3. The ratios of iron to manganese in the nutrient substrate corresponding to good growth and development of plants free from pathological symptoms fluctuated within a narrow range around 2.0, regardless of the total concentrations of these elements within the limits employed. Iron to manganese ratio values above this effective range invariably produced a specific type of chlorosis resulting from excess iron, or deficient manganese, or both; ratio values below this effective range invariably produced a specific chlorosis of a different type resulting from excess manganese, or deficient iron, or both.

4. Ratios of (soluble) iron to (soluble) manganese, within the tissues, associated with good growth and development of the plants cover the same range of values as do those in the external medium, although the range of effective values may be somewhat wider in the plant tissues than in the substrate. Variation in the ratios of the soluble fractions either above or below this effective range, resulted in specific pathological symptoms.

5. High concentrations of soluble manganese in the tissues are invariably associated with low concentrations of soluble iron and vice versa. This suggests the oxidation of ferrous to ferric ions by active manganese, resulting in the inactivation and precipitation of iron in the form of ferric organic complexes.

6. Iron and manganese are definitely interrelated in their metabolic functions, the biological effectiveness of the one being determined by the proportionate presence of the other.

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INFLUENCE OF SUBMERSION OF THE ROOTS ON TRANSPIRATION, APPARENT PHOTOSYNTHESIS, AND RESPIRATION OF YOUNG APPLE TREES

N. F. Childers and David G. White

(WITH NINE FIGURES)

Introduction

In spring there are often places in the orchard where water stands beneath the trees for a week or more. Within a short time the water recedes and the soil appears to be properly drained for the remainder of the season. The question arises as to the importance to the tree of these short periods of excess-soil-moisture conditions, especially when the tree is in leaf. Experiments are described in this paper in which transpiration, apparent photosynthesis and, in some cases, respiration of young apple trees were determined before and after the roots were submerged. In several experiments the determinations were continued after the excess water had been drained from the soil in order to ascertain how soon, if ever, the leaves recovered in activity.

The literature pertaining to root submersion necessarily includes many studies in soil aeration. The well-known monographs by Clements (**6**) in 1922, and Cannon (**3**) in 1925, summarize the work to that period. Certain symptoms which accompany poor root aeration of mesophytes are common knowledge. It is known that poor aeration will cause the foliage to wilt, turn yellow, and die; the roots to develop slowly, become darker in color, and finally decompose; and the soil complex becomes depleted in oxygen and higher in carbon dioxide. A proper aeration of the medium tends to correct these faults (**1, 5, 6, 7, 11**).

Materials and methods

The Stayman Winesap variety was selected for these experiments because of its commercial importance and because it seems to be relatively susceptible to the effects of soil water-logging. The shoots used were grown from two- and three-year stocks grafted on French Crab roots. Terminal buds had set on all trees by the time tests were made, with the exception of those shoots used in experiment VIII. The trees were potted in five-gallon crocks or slightly larger wooden tubs and were grown in the greenhouse until placed under test. A loam topsoil taken from an orchard was used for potting. No attempt was made, however, to correlate the soil type with the effects of root submersion. The term excess water, as used in this paper, infers that water constantly stood above the soil surface. Check trees were watered daily, or once every two days, in order to maintain the soil near

field capacity. The HEINICKE-HOFFMAN (10) apparatus was employed for measuring the rates of transpiration and apparent photosynthesis or respiration. Each determination lasted for a period of two and one-half hours.

Four series of experiments were performed in the environment-control chamber (4) on a total of ten trees, and two series were performed outside the greenhouse on a total of five trees. The temperature in the chamber was nearly constant at levels of 80° or 85° F. The vapor pressure was maintained near 16 mm. of Hg. Light averaged from 2,000 to 5,000 foot candles at the surface of the leaves, depending on their distance from the source. The temperature and relative humidity outdoors were averaged

TABLE I

INFLUENCE OF ROOT SUBMERSION ON APPARENT PHOTOSYNTHESIS AND TRANSPIRATION OF STAYMAN WINESAP APPLE LEAVES. EXPERIMENT 8

DATE, 1941	APPARENT PHOTOSYNTHESIS				TRANSPIRATION			
	CO_2/100 CM.²/HOUR		EXPECTED RATE	PERCENTAGE OF EXPECTED RATE	H_2O/100 CM.²/HOUR		EXPECTED RATE	PERCENTAGE OF EXPECTED RATE
	TEST	CONTROL	$\frac{A}{B} \times 100$	$\frac{C}{78.5^*} \times 100$	TEST	CONTROL	$\frac{E}{F} \times 100$	$\frac{G}{85\dagger} \times 100$
	A	B	C	D	E	F	G	H
March 20	5.76	7.62	76	97	0.85	0.99	86	101
21	11.36	13.78	82	104	1.05	1.23	85	100
22	10.94.	13.60	80	102	0.84	0.97	87	102
23	11.08	14.63	76	97	0.82	0.99	83	98
	ROOTS SUBMERGED							
26	9.94	13.90	72	92	0.91	0.91	100	118
28	9.79	12.19	80	102	0.76	0.88	116	136
30	11.42	13.78	77	98	0.99	1.04	95	112
April 1	13.43	16.05	84	107	1.11	1.29	86	101
3	13.75	17.79	77	98	1.08	1.28	84	99
4	12.82	15.82	81	103	1.01	1.09	93	109
6	11.43	13.78	83	106	0.94	1.32	71	84
8	14.67	14.67	100	127	0.84	0.87	97	114
10	12.28	13.94	88	112	0.87	0.84	104	122
14	11.09	14.78	75	96	0.89	0.87	102	120
16	8.33	13.20	63	80	0.69	0.70	99	116
18	8.19	12.07	68	87	0.58	0.71	82	96
20	5.92	11.72	51	65	0.59	0.69	86	101
22	4.80	13.78	35	45	0.41	0.80	51	60
24	3.08	14.31	22	28	0.33	0.73	45	53
26	2.47	13.42	18	23	0.25	0.75	33	39
28	0.35	11.42	3	4	0.13	0.75	17	20
30	1.92	12.09	16	20	0.12	0.70	17	20
May 2	0.00	11.38	0	0	.....	.....	.....	.....

* Average (78.5) of expected rate.
† Average 85.0 of expected rate.

from three measurements at the beginning, middle, and end of each determination. A sling psychrometer was used to determine the dew point which was then converted to vapor pressure by use of psychrometric tables (**13**). The average foot candles of light outside was calculated from the records of a Micromax continuous recorder connected with a photoelectric cell in the immediate vicinity of the trees. Supplementary experiments, described later, were arranged in the greenhouse to make measurements of leaf petiole angles and to afford observation of root growth.

Method of calculation

The percentage of the expected rate of apparent photosynthesis was calculated as follows: The average milligrams of carbon dioxide absorbed by the proposed test leaves was divided by the average milligrams absorbed by the check leaves. The quotients multiplied by 100 express the percentage relationship between test and check leaves. The average of these percentages found before treatment began was then considered as 100 per cent., or that rate which the proposed test leaves would be expected to maintain if they were not affected by the treatment. Thereafter, the percentage relationships between test and check leaves were divided by the average percentage relationship of the pre-treatment period and multiplied by 100. These products are plotted as percentages of expected rates for each experiment. A typical set of data is presented in table I. Respiration and transpiration were calculated in a similar manner.

Discussion of results

Descriptions of trees used in chamber

Symptoms of leaf injury which accompanied submersion of the roots appeared on the lower leaves first and progressed acropetally. Abscission of two or more lower test leaves often occurred before termination of an experiment. In experiment I the leaves on the test tree developed light green areas between the veins. The foliage wilted and drooped mostly from the distal ends of the petioles. At the end of experiment IV the leaves on test trees showed only a greater devlopment of anthocyanin pigments as compared with the check leaves. No apparent differences in the foliage occurred between the test trees and controls in experiment V. The leaves on the test trees in experiment VIII developed light green areas between the veins. The foliage wilted and drooped (fig. 1) and finally appeared similar to the treated tree in experiment I.

Descriptions of trees used outdoors

Considerable tip and marginal burning had occurred within eight days after flooding the roots of one tree outside in July (experiment II). The

leaves on a second tree of the same experiment only wilted and drooped, similar to the foliage of the test tree in experiment I in the control chamber. The leaves on trees having roots submerged from late September to late October, experiment III, were slower in developing injury, probably because of the cooler autumn temperatures. The lower leaves showed yellowish-red

FIG. 1. Roots of the tree on the left were submerged in water for 45 days in the environment-control chamber. Tree on the right was watered every second day. Both trees were of equal vigor at the outset. See figure 8 for comparison of transpiration and apparent photosynthesis.

splotches which finally became necrotic. The blades curled upward at the margins but did not droop. At the end of the experiment the upper leaves appeared similar to the upper leaves of the check tree except for the presence of considerable anthocyanin pigments. The symptoms of leaf injury which appeared in these experiments were similar to descriptions given by HEINICKE, *et al.* (**9**) for orchard trees purposely flooded.

EXPERIMENT I. PHOTOSYNTHESIS AND TRANSPIRATION

Experiment I was conducted in the chamber at a temperature of 85° F. Six comparable leaves on each of two trees were selected for study. The roots of one tree were constantly submerged beginning June 22, 1940. The percentages of the expected rates of apparent photosynthesis and transpiration are plotted in the upper graph of figure 2. The points on the chart

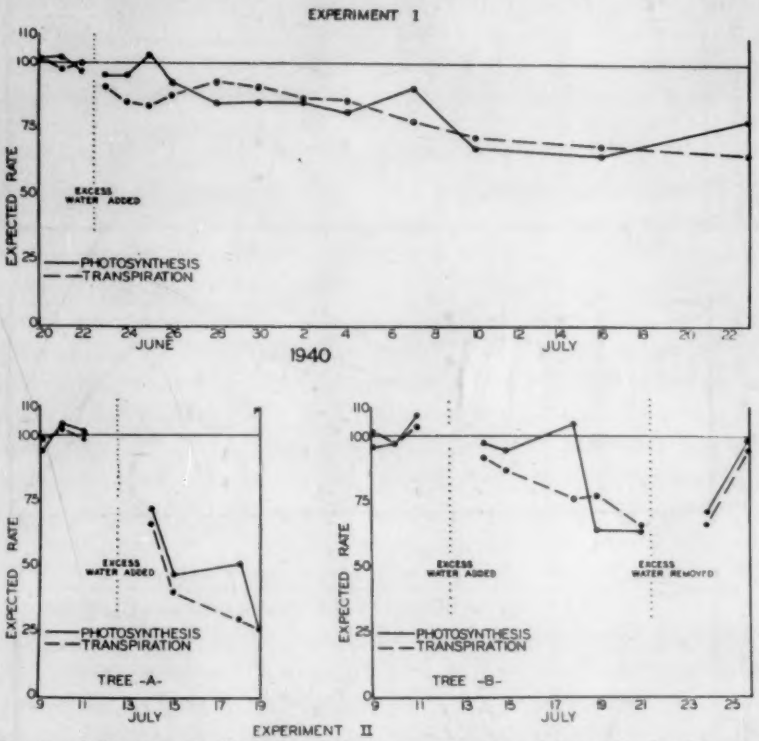

FIG. 2. Influence of submersion of roots on apparent photosynthesis and transpiration of apple leaves. Expressed as percentage of expected rate. Horizontal line at 100 represents photosynthesis and transpiration of check leaves. Experiment I was performed in environment-control chamber; experiment II was out-of-doors.

during the pre-treatment period exemplify the usual variation in activity of the test leaves as compared with the check leaves. Both apparent photo-

synthesis and transpiration gradually decreased to between 80 and 70 per cent. within the 30-day period of submersion.

Experiment II. Photosynthesis and transpiration

Experiment II was performed on two test trees and a check tree placed outside of the greenhouse in July, 1940. Four comparable leaves were used on each tree. The results are shown as percentages in the lower two graphs of figure 2. The rates of photosynthesis and transpiration of test tree A had decreased to 25 per cent. seven days after the flooding period started on July 13. Extreme foliage injury occurred and prevented further measurements

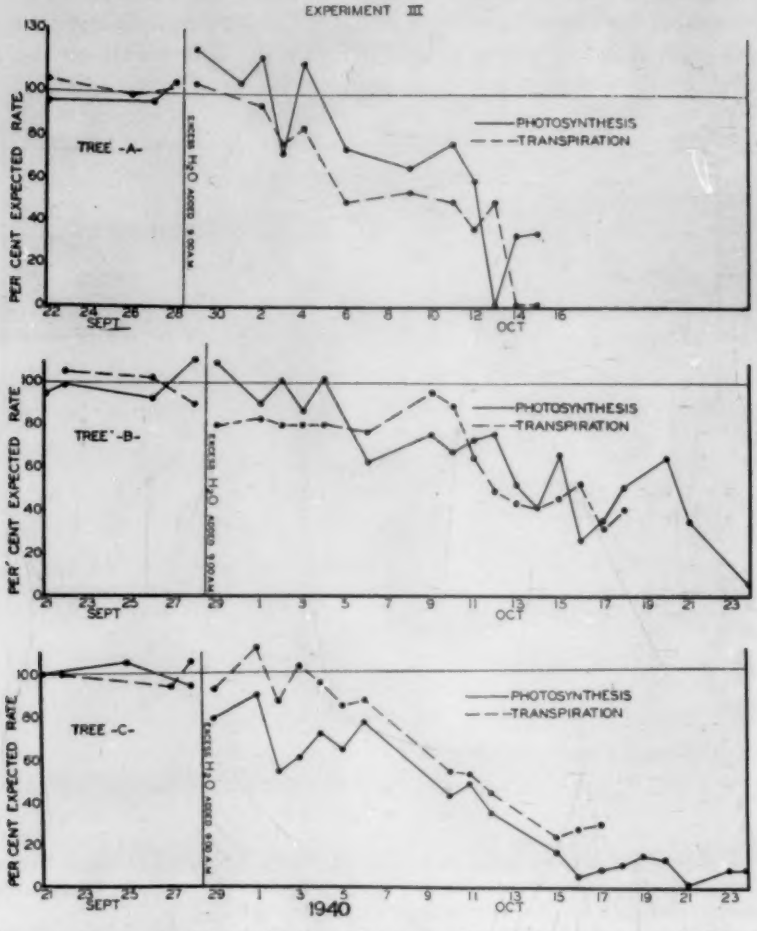

Fig. 3. Apparent photosynthesis and transpiration of trees A, B, and C in experiment III. Note differences in individual tolerance of trees to root submersion. Experiment performed out-of-doors.

on this tree. The apparent rates of photosynthesis of test tree B decreased to 67 per cent. during the same seven days of submersion, while transpiration decreased to 69 per cent. The soil of tree B was drained of free water after nine days of submersion; six days later the rates of leaf activity of the test tree were equivalent to its pre-treatment period.

Experiment III. Photosynthesis and transpiration

Experiment III was conducted outside the greenhouse during late September and through most of October, 1940. The roots of three trees were submerged on September 29 and a fourth tree was used as a check. Three comparable leaves were used on each tree. The percentages of expected rates of apparent photosynthesis and transpiration are given in figure 3. Both processes ultimately were severely reduced, with photosynthesis less than 10 per cent. of its expected rate. The time of lowest leaf activity varied with individual trees. The roots of the trees were washed free of soil at the end of the experiment. Several short, rather stubby, white roots had developed on or near the graft union of the flooded trees.

Experiment IV. Photosynthesis, transpiration, and stomatal activity

Experiment IV consisted of two test trees and a check placed in the chamber at 80° F. Four leaves on each tree were used; the results are plotted in figure 4. As much as a 96 per cent. reduction in apparent photosynthesis of test tree A and a 79 per cent. reduction in test tree B were associated with submersion of the roots. Transpiration rates were reduced also but not so severely. The free water was drained after 18 days of flooding, and within 4 to 6 days there was a trend toward recovery in leaf activity. The leaf activity of tree A did not completely recover by the end of the experiment. Transpiration of tree B evidently recovered while photosynthesis continued between 85 and 100 per cent. of its expected rate. The roots were washed free of soil at the end of the experiment, 14 days after the excess water had been drained. Roots of the test trees were scanty as compared with those of the check tree. The test roots were also darker in color than the check roots; no other differences were visible.

In experiment IV stomatal activity of the leaves under study was observed through a Leitz "Ultrapak" microscope. The study was made on November 22, 23, and 24, when leaf activity had become low owing to extended root submersion. The counts recorded in each observation were an average from a part of three fields near the apex of a leaf. Each count included an observation of 25 to 40 stomata. Any stoma which did not appear to be completely closed was recorded as open. No attempt was made to note differences in degree of opening since there appears to be no positive method of knowing when a stoma is partly open or closed. The data are

plotted in figure 5 as percentage of stomata open. There were no consistent differences between the activity of stomata of treated and check trees. Hence, the marked reductions in photosynthesis and transpiration (fig. 4) could not be correlated with stomatal activity during this 3-day period.

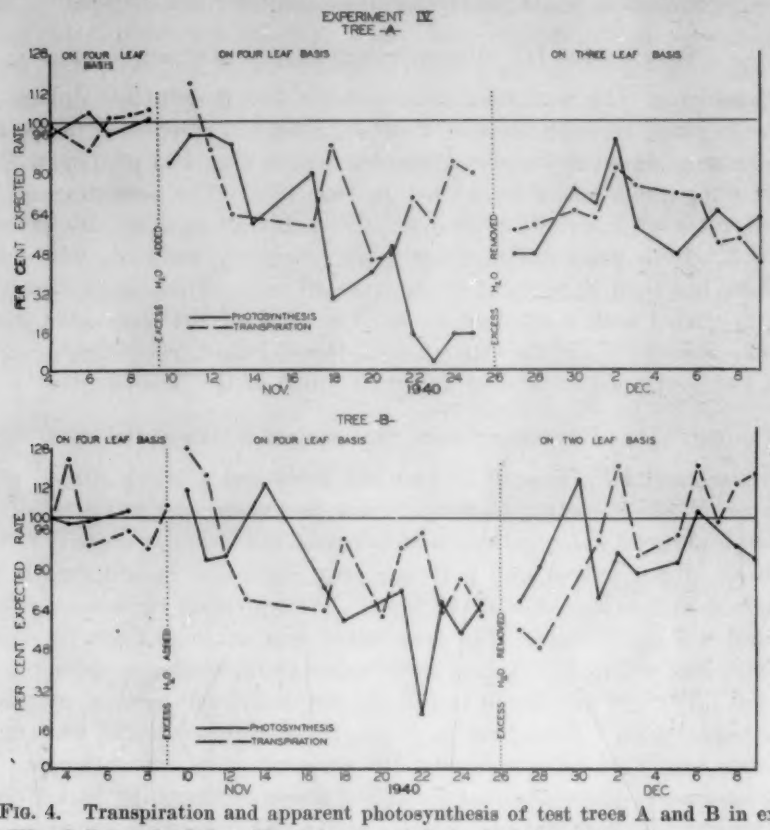

FIG. 4. Transpiration and apparent photosynthesis of test trees A and B in experiment IV. Soil was flooded on November 9 and drained 17 days later.

EXPERIMENT V. PHOTOSYNTHESIS, TRANSPIRATION, RESPIRATION, AND LEAF TEMPERATURE

Experiment V was conducted with two trees in the chamber at a temperature of 85° F. This experiment was designed to measure any differences in the activity of upper leaves as compared with middle leaves on a shoot with submerged roots. The experimental leaves were large and mature. A few of the basal leaves of each tree yellowed and abscised soon after being placed in the chamber. Three of the top-most leaves which would fit the leaf-cups were used to compare with three leaves located approximately at the middle of the shoots. The shoots were about five feet in height. Ap-

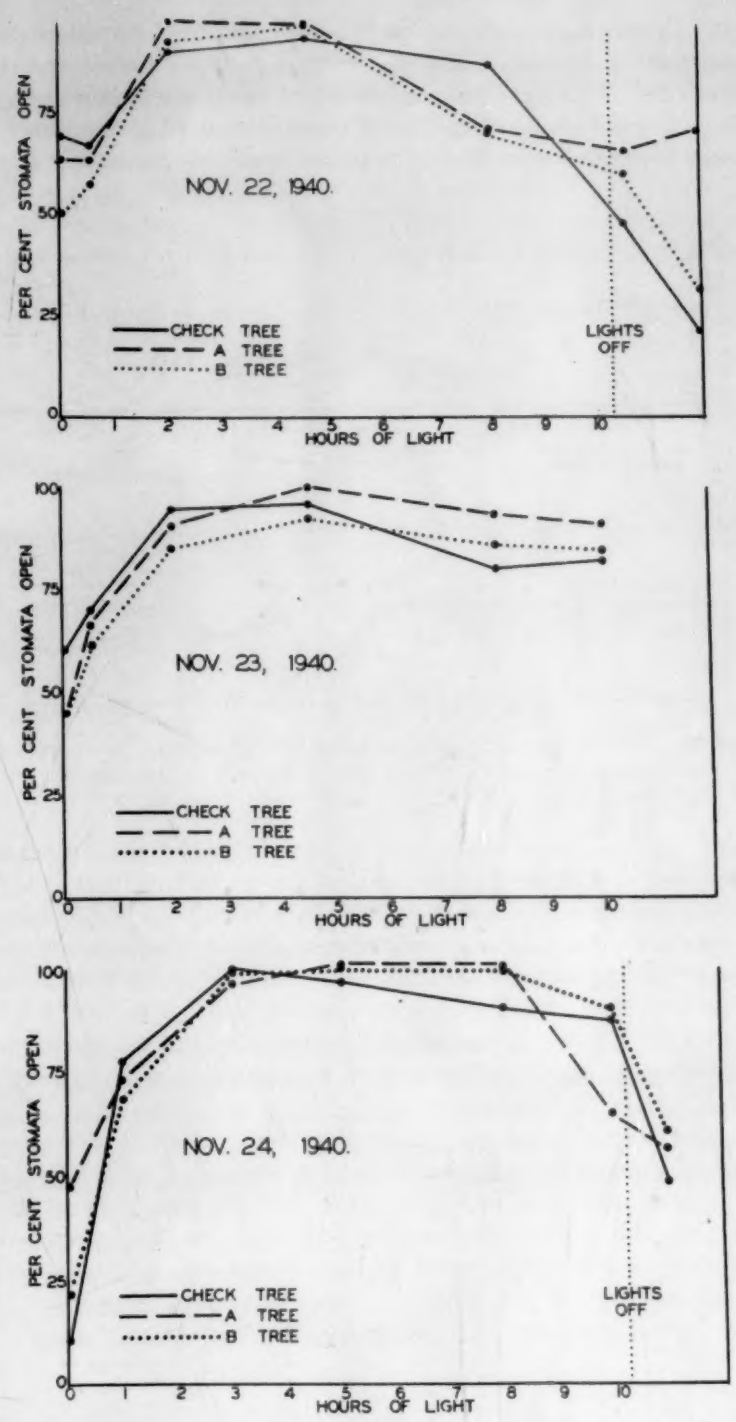

FIG. 5. Stomatal behavior of test and control leaves from November 22 to 24, inclusive, experiment IV. During this period apparent photosynthesis and transpiration were showing reductions of 20 to 95 per cent., 13 days after beginning submersion.

parent photosynthesis and respiration were measured on alternate days. The respiration determinations began about 6:00 P.M., when the chamber was darkened. Transpiration was measured in all determinations.

The apparent photosynthesis and transpiration of the test tree in experiment V are shown in figure 6. In the upper leaves the trends of both

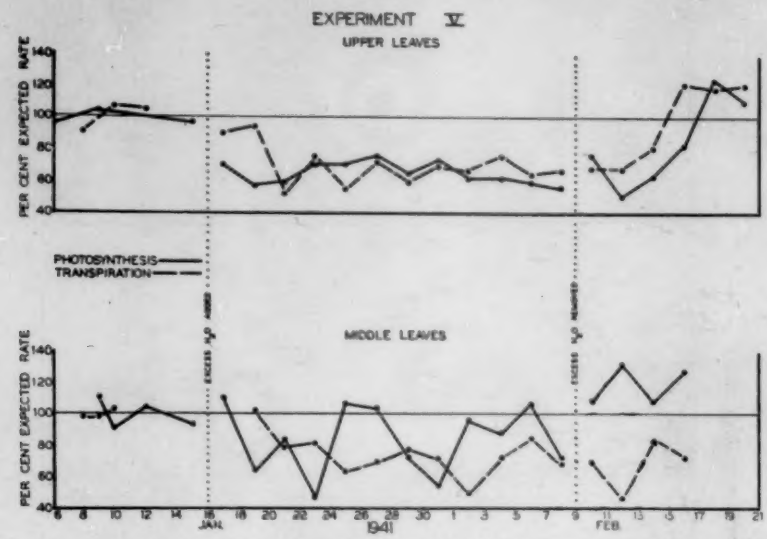

FIG. 6. Transpiration and apparent photosynthesis of upper and middle set of leaves on one-year tree in experiment V. Roots were submerged on January 16; water was drained on February 9.

processes were similar, showing 30 to 50 per cent. reductions throughout the 25-day period of submersion. Removal of the excess water was followed by apparent recovery in leaf activity. The photosynthesis of middle leaves was not consistently less than the expected rate after submersion, although an average of the twelve determinations for the period would fall well below the 100 per cent. line. Nevertheless, it appears that photosynthesis of the middle set of leaves was not affected as much as that of the upper set. Transpiration of middle leaves showed a reduction similar to that which occurred in the upper leaves.

Respiration and transpiration, as determined at night, are plotted in figure 7. It should be emphasized that the amount of carbon dioxide released by the leaves was relatively small, and the possibility of error in procedure could be somewhat greater. As shown in the graph, however, the trends were reasonably consistent from day to day. Apparent respiration after submersion was definitely increased in both upper and middle leaves. Indeed, on some days respiration of test leaves was almost double

the expected rate. The decrease in respiration which occurred after 10 to 14 days of submersion may have been caused by smaller amounts of carbohydrates present in the leaves, which is probable since there was a lower rate of photosynthesis by that time. Transpiration of middle leaves was reduced more than in the upper leaves, and recuperation did not occur within nine days after drainage as it did in the upper leaves.

Leaf temperatures were determined for the leaves used in experiment V. Thermocouples of copper and constantan wires, no. 30 B. and S. gauge, were

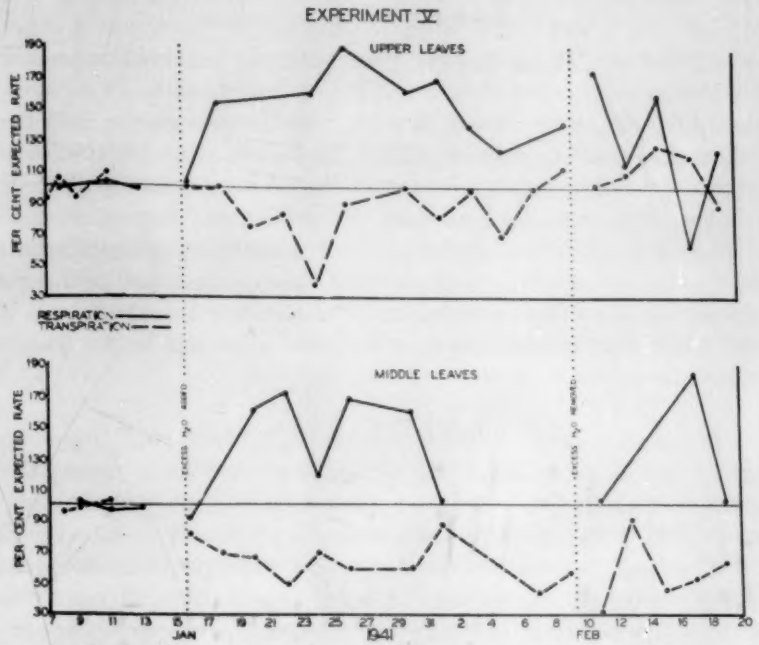

FIG. 7. Transpiration and apparent respiration of the upper and middle set of leaves on test tree in experiment V. Note the increased rate of respiration which occurred after submersion.

constructed in the glass leaf cups similar to the method employed by WAUGH (**14**). The wires were arranged so that their own tension gently pressed the thermocouple against the under side of the leaf on approximately the same area each time. The leads from the cups in the chamber were attached to a multiple switch outside. A Leeds and Northrup potentiometer was employed to measure the temperature of each leaf and the air in degrees F. The apparatus was accurate to about 1°. Readings were made after the photosynthesis or respiration determination had been in progress about an hour. It was made certain that the cooling unit in the chamber had not been operating for at least fifteen minutes before the leaf temperatures

were determined. The current cold weather made it unnecessary to operate the cooling unit at night. There were no significant differences between the leaf temperatures of the test and check trees during the determinations in either light or dark. The average temperature of the upper leaves was higher than that of the middle leaves during photosynthesis determinations because of their proximity to the light source. During dark periods this difference did not occur. At night, however, in all cases, the leaf temperatures were 1½ to 4 degrees cooler than the surrounding air.

Experiment VI. Leaf angles

In experiment VI an ordinary flat compass was used to measure at weekly intervals the angle of each leaf petiole to the shoot. Two trees, with terminal buds set, were chosen to remain in the greenhouse, and the root system of one tree was submerged for 78 days. Although the usual leaf discolorations accompanied submersion, there was no consistent change in leaf angles. This was also a common characteristic of several other treated trees, especially when the foliage was well matured. In experiment VIII, figure 1, a change occurred when the relatively young leaves assumed somewhat wider angles after submersion. The test tree in experiment VI developed a new root system immediately below the water level. These roots arose from the crown and were well branched but did not possess root hairs.

Experiment VII. Root growth

The object of experiment VII was to trace the daily development of small apple roots before and after submergence. Six dormant one-year apple seedlings were planted in the greenhouse on March 14 in two rectangular boxes having glass sides. Wooden covers were placed against the sides of the boxes between observations in order to prevent light from reaching the roots which grew against the glass. As each new root tip appeared it was numbered on the glass with a wax pencil at its place of origin. Linear measurements of the visible roots (totaling about 300) were made throughout the experiment.

Root development was first evident five days after planting, although no foliage had yet opened. Some roots produced an abundance of root hairs but others had none. A new root tip usually elongated 2 to 4 millimeters a day and in rarer cases as much as 1 centimeter. These roots were from 1 to 1.5 millimeters in diameter, and at the time of their death varied in length from a few millimeters to 11 centimeters. Although conditions were favorable for root growth, the average length of life of the visible roots was usually only about one week. There were generally 15 to 25 live roots visible at any one time; in other words, more new roots appeared at about the same rate as others were dying. The rootlets soon disintegrated after

dying. Sometimes short branch roots arose from a vigorous new root, but later the side roots died and were followed by the disintegration of the parent root.

It is assumed that the roots did not die because of any unfavorable condition at the glass since this same condition probably would have prevented the formation of new roots. These observations agree with the report by KOLESNIKOV (**12**), who found that death of the root tips of fruit trees ordinarily occurs even under favorable conditions. The deaths of whole rootlets, and often of small sub-systems, were noted to a considerable extent over the entire main root system. HARRIS (**8**) observed that a rise of the water table in autumn was associated with cessation of apple root growth, with eventual death to newly formed roots.

In this experiment submersion inhibited the formation of new roots but did not cause an immediate reduction in the linear growth of roots already present. Such results are in agreement with BOYNTON (**2**) who concluded that a higher level of oxygen may be necessary for the production of new rootlets than for the maintenance of existing roots. After 18 days of submersion all visible roots were dead when the free water was drained. Within 8 days after drainage new root tips were evident close to the soil surface, and within two weeks new root tips were apparent at all depths.

EXPERIMENT VIII. PHOTOSYNTHESIS AND TRANSPIRATION

Experiment VIII was conducted with two trees in the chamber at 85° F. The shoots were about 3 feet in height and still growing. At the beginning of the experiment the tips of both shoots were placed the same distance from the light source by raising one crock. Air was drawn through a pliofilm hood placed over the tip of each shoot during the determinations. By this method the activity of the entire end of the shoot was measured, including a dozen or more mature and young leaves. The leaves were traced each day and their respective areas determined with a planimeter.

The roots of one tree were submerged March 24 and thereafter photosynthesis determinations were made every other day. Forty days of submersion severely injured the test tree as shown in figure 1. At the end of the experiment leaves which had been under the hoods were removed and their fresh weights were determined. The test leaves weighed 0.75 gm. and check leaves 2.50 gm. per 100 square centimeters of surface. The same leaves were next placed in an oven at 103° C. for 48 hours. Test leaves were found to contain 18 per cent. water as compared with 75 per cent. in check leaves. Expressed on the leaf area basis, the oven dry test leaves weighed 0.616 gm. and the check leaves 0.659 gm. per 100 sq. cm. The dry leaves were carefully ashed for 24 hours in a muffle furnace at 650° C. The ash of the test leaves was equivalent to 22 mg. and that of the check leaves 32 mg. per 100 sq. cm. of surface.

Leaf activity of the trees used in experiment VIII is shown in table I and figure 8. Photosynthesis was not reduced definitely until after three weeks of submersion. Yet the initial relationship between total leaf areas of test

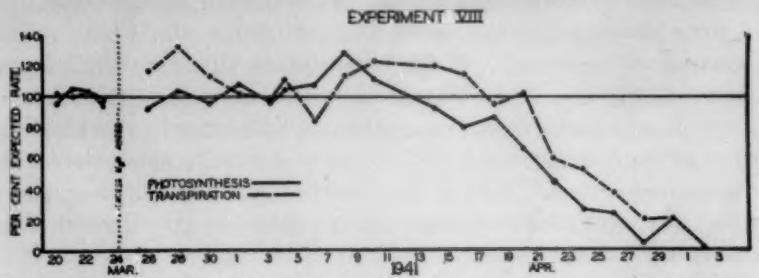

FIG. 8. Transpiration and apparent photosynthesis of test tree in experiment VIII. See figure 9 for rate of increase in leaf area of these leaves.

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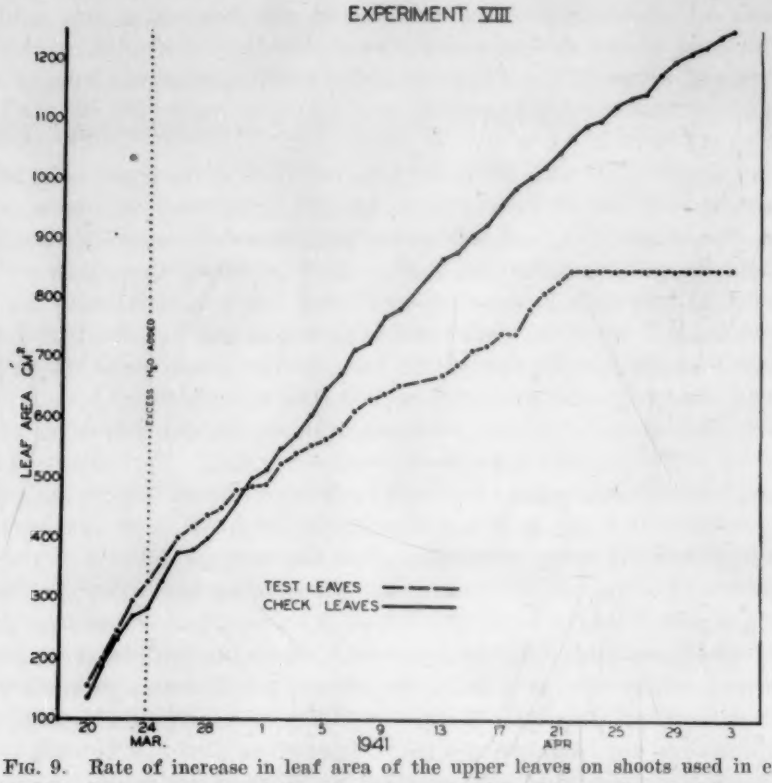

FIG. 9. Rate of increase in leaf area of the upper leaves on shoots used in experiment VIII. Note change in relationship between growth of test and control leaves after test roots had been submerged for one week, March 24 to April 1.

three weeks of submersion, and was slower in showing a definite reduction. Therefore, it appears that an inhibition of the growth of tip leaves may give evidence of water-logging injury before there are definite reductions in metabolism per unit area of the mature and young leaves on a shoot.

Summary

1. The roots of young apple trees growing in soil in 5-gallon containers were submerged in water for a week to two months, then drained. Transpiration, apparent photosynthesis, and respiration were measured with the HEINICKE-HOFFMAN apparatus, before, during and after submergence of the roots. Experiments were performed both in the field and in an environment-control chamber.

2. Transpiration and apparent photosynthesis were reduced within 2 to 29 days, usually within 2 to 7 days, after the roots were submerged. In some cases the rates of transpiration and apparent photosynthesis became so small with continued submersion that they could not be measured.

3. Apparent respiration was increased within 2 days after submerging the roots. When water was drained from the soil, the rate of respiration returned to near the pre-treatment rate.

4. Leaf temperature and the behavior of stomata could not be correlated with low leaf activity due to root submergence.

5. Leaves from submerged trees contained less water and ash per unit of leaf surface than leaves from check trees.

6. Small apple roots were observed to die even under favorable soil conditions, but the development of new roots and formation of root hairs were inhibited by submergence.

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GROWTH RATES OF MAIZE UNDER FIELD CONDITIONS[1]

ROY A. BAIR

(WITH SIX FIGURES)

Introduction

The present study was undertaken during the growing seasons of 1938 and 1939 to measure weekly changes in total dry weight of four varieties of maize grown on the agronomy farm of Iowa State College at Ames, Iowa. The purposes of this investigation were: (1) to compare the growth curves of early and late maturing varieties in a given complex of climatic and edaphic conditions; (2) to make comparisons between the performance of these varieties in two different years; (3) to ascertain whether dry weight increment varied significantly from week to week, particularly during the main period of growth; and (4) to attempt to correlate any incremental variations with climatic fluctuations.

Various measures of growth have been reported in the literature. Attempts to describe the growth curve or to evaluate the effect of an environmental factor on plant development have been based on increase in dry weight or in leaf area, and on rate of elongation. Dry weight has been used for entire plants, aerial parts only, leaves, and fruit only. Increase in size has been measured as total plant height or has utilized various plant parts such as roots, stems, leaves, or seedling structures.

Using these measurements, many investigators have attempted to give mathematical expression to the growth curve of plants. The similarity between the calculated and observed growth values has led REED and HOLLAND (**12**), MURNEEK (**10**), PEARL *et al.* (**11**) and others to emphasize the control of the growth rate by constant internal factors rather than by external conditions. It was pointed out by GREGORY (**6**), however, that the rate of growth is conditioned by the state of the plant which, in turn, is conditioned both by previous history and the present general environment. EISELE (**5**) reported that the weekly increase in dry weight of maize plants followed the autocatalytic type of curve for single-plant hills, but that the curve was more nearly straight in crowded plantings. Calculating data for maize plants as increase in dry weight per square meter of leaf area per week, BRIGGS *et al.* (**3**) found unit leaf rate to be correlated more closely with temperature than with any other environmental factor.

[1] Journal paper no. J-984 of the Iowa Agricultural Experiment Station, Ames, Iowa, project no. 610. Cooperative investigation between the Agricultural Marketing Service, U. S. Department of Agriculture, and the Iowa Experiment Station.

Leaf activity of the trees used in experiment VIII is shown in table I and figure 8. Photosynthesis was not reduced definitely until after three weeks of submersion. Yet the initial relationship between total leaf areas of test

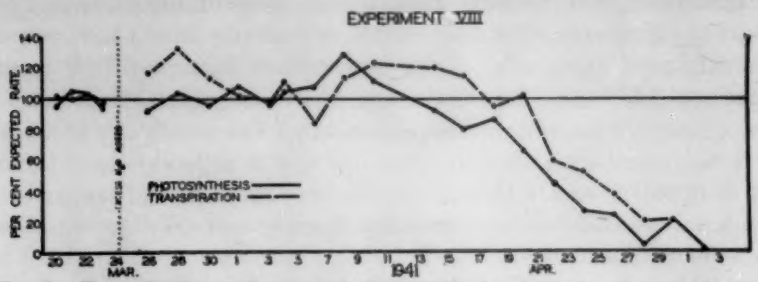

FIG. 8. Transpiration and apparent photosynthesis of test tree in experiment VIII. See figure 9 for rate of increase in leaf area of these leaves.

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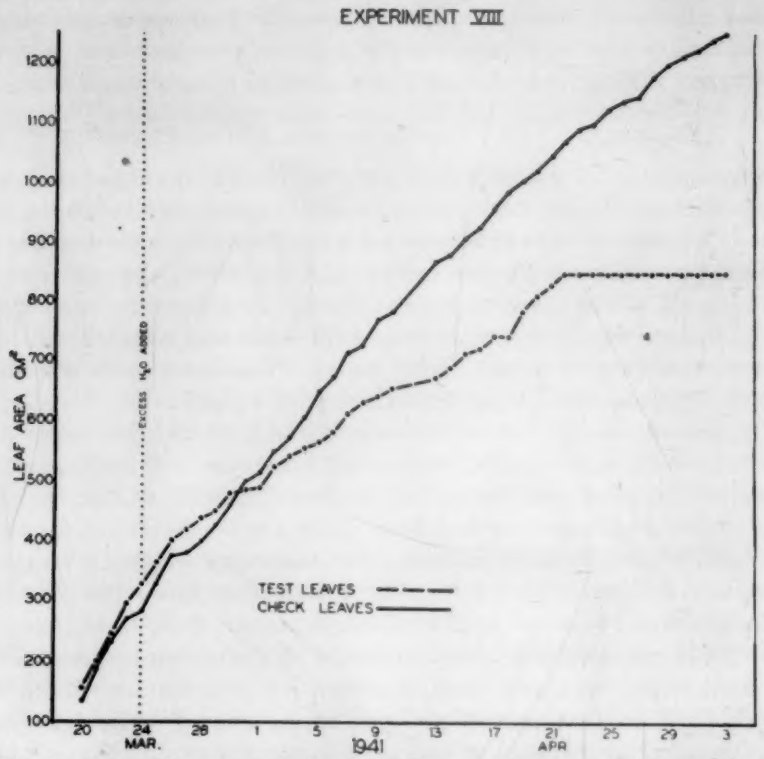

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Introduction

The present study was undertaken during the growing seasons of 1938 and 1939 to measure weekly changes in total dry weight of four varieties of maize grown on the agronomy farm of Iowa State College at Ames, Iowa. The purposes of this investigation were: (1) to compare the growth curves of early and late maturing varieties in a given complex of climatic and edaphic conditions; (2) to make comparisons between the performance of these varieties in two different years; (3) to ascertain whether dry weight increment varied significantly from week to week, particularly during the main period of growth; and (4) to attempt to correlate any incremental variations with climatic fluctuations.

Various measures of growth have been reported in the literature. Attempts to describe the growth curve or to evaluate the effect of an environmental factor on plant development have been based on increase in dry weight or in leaf area, and on rate of elongation. Dry weight has been used for entire plants, aerial parts only, leaves, and fruit only. Increase in size has been measured as total plant height or has utilized various plant parts such as roots, stems, leaves, or seedling structures.

Using these measurements, many investigators have attempted to give mathematical expression to the growth curve of plants. The similarity between the calculated and observed growth values has led REED and HOLLAND (**12**), MURNEEK (**10**), PEARL *et al.* (**11**) and others to emphasize the control of the growth rate by constant internal factors rather than by external conditions. It was pointed out by GREGORY (**6**), however, that the rate of growth is conditioned by the state of the plant which, in turn, is conditioned both by previous history and the present general environment. EISELE (**5**) reported that the weekly increase in dry weight of maize plants followed the autocatalytic type of curve for single-plant hills, but that the curve was more nearly straight in crowded plantings. Calculating data for maize plants as increase in dry weight per square meter of leaf area per week, BRIGGS *et al.* (**3**) found unit leaf rate to be correlated more closely with temperature than with any other environmental factor.

[1] Journal paper no. J-984 of the Iowa Agricultural Experiment Station, Ames, Iowa, project no. 610. Cooperative investigation between the Agricultural Marketing Service, U. S. Department of Agriculture, and the Iowa Experiment Station.

Methods

Adjacent plots of approximately one acre each (260 by 180 feet) were used, one each year. These plots were part of a block in which a three-year wheat-alfalfa-maize rotation had been followed. It was considered that the variability between years would be less for changed plots on fairly homogeneous locations than for successive plantings of maize on the same soil. The soil ranged from Clarion loam to Webster silt loam in the 1938 plots, and from Clarion loam to Webster silty clay loam in the location used in 1939. The

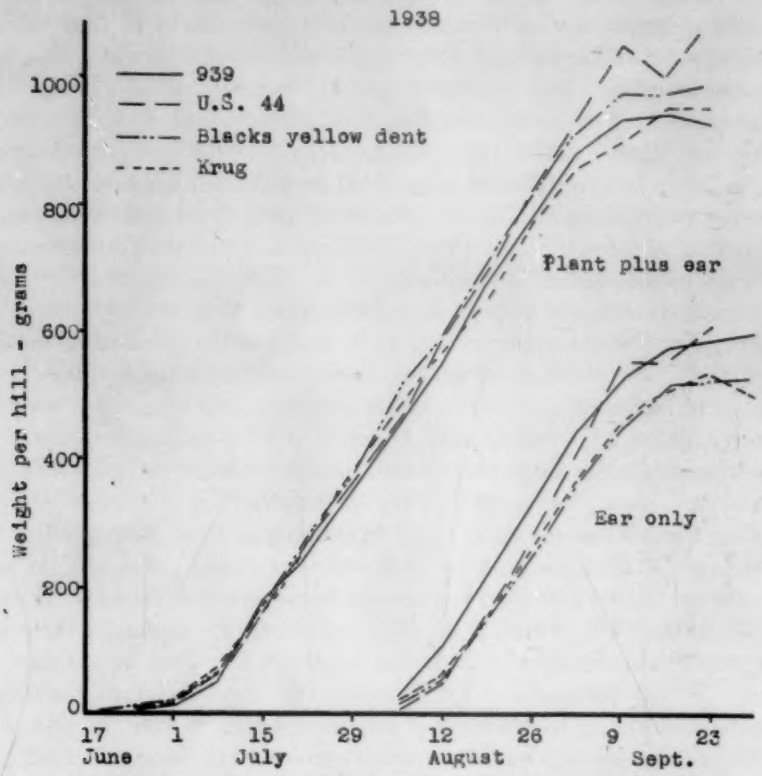

FIG. 1. Weekly means of total dry weights per hill of four varieties of maize, growing season of 1938.

proportion of Clarion to Webster was approximately the same in the two locations. A more detailed description of these soils has been presented elsewhere (**13**).

In each year four varieties of maize were planted. Two (Blacks yellow dent and Krug) were open pollinated; and two (Iowa 939 and U. S. 44) were hybrid. Iowa 939 is a short-season variety while U. S. 44 is late maturing. The planting dates were May 24, 1938, and May 8, 1939. All varieties were planted six kernels to the hill, and the seedlings thinned to three plants in

hills 40 inches apart each way. Each variety was planted in plots of four rows 102 hills long with five replications. The plots in each replication were randomized.

In the sampling program, every plot, consisting of four rows 102 hills long, was divided into two sections of 51 hills each. Samples for dry weight determinations were taken only from the middle two rows of the plot. A

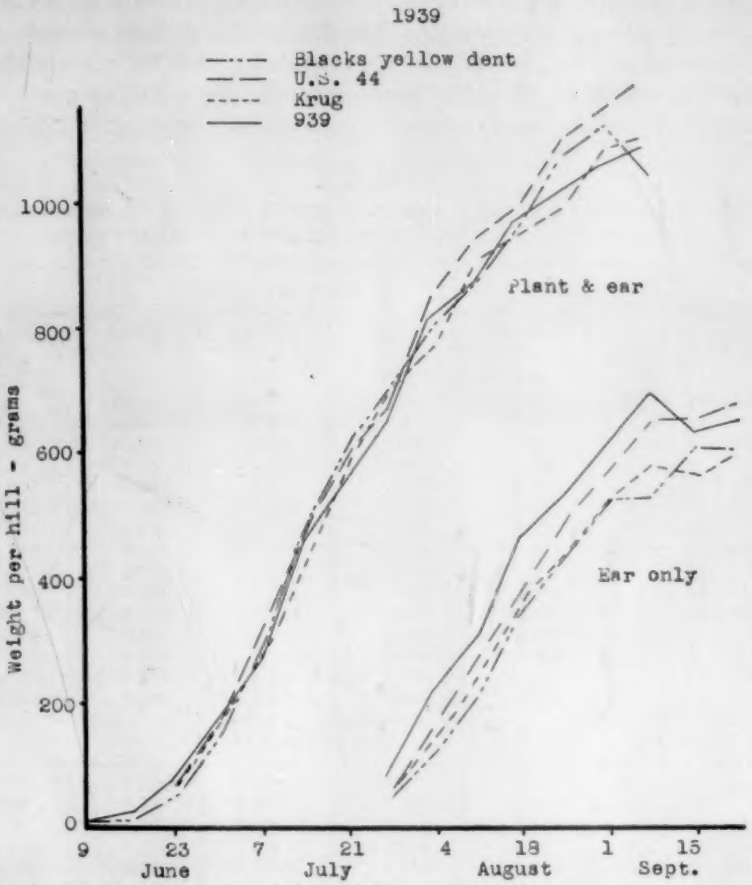

FIG. 2. Weekly means of total dry weights per hill of four varieties of maize, growing season of 1939.

sampling unit consisted of four adjacent 3-plant hills (a total of 12 plants) surrounded by complete hills. Seventeen such sampling units were thus possible in each half-plot. At the beginning of the growing season a sampling program was planned, by drawing numbers from TIPPETT'S tables of randomized numbers (**14**) to provide for the weekly cutting of one unit for each half-plot in every replication of all varieties. The two 12-plant units, one

from each half-plot, constituted the plot sample. Since there were five replications, five such samples of 24 plants each were harvested weekly from each variety for a total of 120 plants per variety.

Each Friday morning during the growing season the plants of the 40 sampling hills were cut just above the surface of the soil and weighed immediately. Four plants taken at random from the 12 in each four-hill unit were finely chopped in a Hobart cutter to provide aliquots of either 100 or 200 grams of green plant material. The dry weight of these aliquots, after 48 hours in an electric oven at 105° C., provided a basis for calculating the original dry weight of the entire harvested unit. In a similar manner the dry weight of the ears was calculated separately each week after silking.

TABLE I

MEAN DRY WEIGHT PER 3-PLANT HILL FROM EACH OF FOUR VARIETIES DURING THE GROWING SEASON OF 1938. EACH MEAN IS THE AVERAGE OF 40 3-PLANT HILLS

DATE	TOTAL DRY MATTER				
	IOWA 939	B.Y.D.	U.S. 44	KRUG	MEAN
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
June 17	0.904	0.804	0.899	0.981	0.874
" 24	6.78	8.22	8.75	6.63	7.60
July 1	19.1	19.3	25.0	20.0	20.8
" 8	62.5	64.5	58.3	62.0	61.7
" 15	181.0	172.0	161.0	165.0	170.0
" 22	259.0	276.0	174.0	279.0	272.0
" 29	360.0	370.0	355.0	369.0	364.0
Aug. 5	454.0	507.0	459.0	486.0	476.0
" 12	554.0	591.0	595.0	572.0	578.0
" 19	680.0	710.0	695.0	653.0	685.0
" 26	779.0	804.0	829.0	770.0	796.0
Sept. 2	883.0	909.0	933.0	859.0	896.0
" 9	930.0	976.0	1051.0	904.0	965.0
" 16	947.0	971.0	1002.0	949.0	967.0
" 23	931.0	990.0	1063.0	954.0	985.0
	DRY WEIGHT OF EAR ONLY				
Aug. 5	28.3	17.7	7.80	17.3	17.8
" 12	108.0	56.1	53.8	64.8	70.8
" 19	228.0	149.0	154.0	156.0	172.0
" 26	330.0	248.0	276.0	262.0	279.0
Sept. 2	446.0	362.0	416.0	363.0	397.0
" 9	527.0	447.0	550.0	454.0	496.0
" 16	569.0	509.0	551.0	506.0	534.0
" 23	591.0	525.0	608.0	529.0	563.0

Results

VARIETAL RESPONSE TO WEATHER

Weekly mean dry weights per 3-plant hill for each of the four varieties are shown for 1938 and 1939 in tables I and II, respectively, and are pre-

sented graphically in figures 1 and 2. The similarities in slopes of the total dry weight curves for the varieties grown each season raised the question as to whether the small weekly variations in rate represented actual differences in varietal response to a given complex of climatic and edaphic conditions, or whether such fluctuations in the curve were the result of sampling variability.

Since the absolute gain in dry weight per week for each of the varieties varied as the season progressed, and since the standard deviations of the dry

TABLE II

MEAN DRY WEIGHT PER 3-PLANT HILL FROM EACH OF FOUR VARIETIES DURING THE GROWING SEASON OF 1939. EACH MEAN IS THE AVERAGE OF 40 3-PLANT HILLS

DATE	TOTAL DRY MATTER				
	IOWA 939	B.Y.D.	U.S. 44	KRUG	MEAN
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
June 9	9.73	10.3	12.0	11.3	10.8
" 16	22.2	17.9	21.0	20.4	20.4
" 23	69.7	58.9	60.9	64.5	63.5
" 30	159.0	150.0	155.0	170.0	159.0
July 7	273.0	292.0	295.0	282.0	286.0
" 14	425.0	426.0	439.0	428.0	429.0
" 21	547.0	556.0	525.0	539.0	542.0
" 28	634.0	660.0	633.0	641.0	642.0
Aug. 4	784.0	763.0	779.0	741.0	767.0
" 11	837.0	801.0	901.0	849.0	847.0
" 18	926.0	934.0	953.0	931.0	936.0
" 25	1001.0	1013.0	976.0	971.0	990.0
Sept. 1	983.0	1058.0	1110.0	1035.0	1047.0
" 8	992.0	982.0	1069.0	1030.0	1018.0
	DRY WEIGHT OF EAR ONLY				
July 28	80.3	42.0	43.8	57.5	55.9
Aug. 4	207.0	122.0	143.0	150.0	155.0
" 11	293.0	195.0	244.0	236.0	242.0
" 18	428.0	329.0	352.0	357.0	367.0
" 25	521.0	414.0	436.0	428.0	450.0
Sept. 1	561.0	495.0	549.0	499.0	526.0
" 8	630.0	498.0	584.0	546.0	565.0
" 15	616.0	588.0	620.0	532.0	589.0

matter determinations were noticeably much larger when the plants became larger, a transformation of the data was necessary before a test of the significance of varietal differences could be made. The analysis of variance, following the use of a logarithmic transformation, is summarized in table III. The date by variety interaction was not significant, the mean square being actually less than that for error each year, indicating that the varieties did not respond differently to the environments of succeeding weeks.

TABLE III

ANALYSES OF VARIANCE OF THE LOGARITHMS OF THE TOTAL DRY WEIGHTS PER SAMPLE OF FOUR VARIETIES OF MAIZE DURING 14 WEEKS OF EACH GROWING SEASON

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE
1938		
Total	279	
Variety	3	0.0081
Replication	4	0.0143
Date	13	17.3
Date by variety	39	0.0024
Error	220	0.0032
1939		
Total	279	
Variety	3	0.0030
Replication	4	0.0062
Date	13	8.76
Date by variety	39	0.0022
Error	220	0.0026

SEASONAL VARIATION IN GROWTH

On the basis of the evidence that the four varieties reacted alike to the climatological complex in any given week, it was considered appropriate to regard them as constituting a homogeneous group during each weekly period. Figure 3 presents the curve for each year when the data for all varieties are pooled, the time being measured in days after planting. Each point on the graph represents a mean obtained from the harvest of 160 hills. As might have been expected, the 1938 crop, which was planted late, developed more rapidly than the 1939 crop, which was planted a full two weeks earlier in the season. This speed is evidenced by a greater total growth in 1938 than in 1939 during the first eight weeks after planting; and also by the fact that the development of the ears was more rapid in 1938.

Similarities in growth curves for the two years are perhaps more striking than the differences; the point at which the curves cross, however, is of particular interest in that it may have presaged the slightly higher yield which was to come in 1939. A test of significance of the year by date interaction for the three dates of each year beginning one week before and ending one week after the point of crossing of the curves, resulted in an F value far beyond the one per cent. point. The evidence is very strong, therefore, that the apparent higher rate of growth for two weeks in 1939, as compared with 1938, resulting in the crossing of the curves at that point, was not due to sampling variation. The 1939 growth curve for total dry weight per hill remained above the 1938 curve for the remainder of the season. In contrast, the curve for weight of ears lagged behind the 1938 curve until the end of

the season, suggesting that the 1939 conditions favored vegetative growth more than fruit development.

Weekly increments in dry weight were calculated each season for plants only, ears only, and for entire plants (figure 4). Here the differences in rate of growth from week to week and for corresponding weeks in 1938 and 1939 assert themselves more clearly. The fact that in 1939 the rate of growth was relatively more rapid during July and declined during August, con-

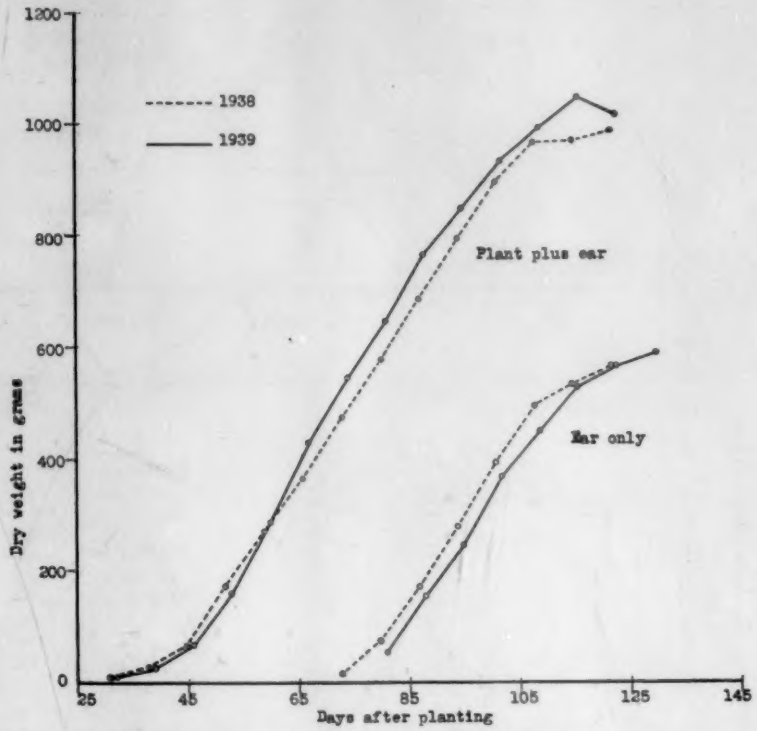

FIG. 3. Weekly means of total dry weights per hill of four varieties of maize in 1938 and 1939. Each point on the curves represents a mean obtained by the harvest of 160 hills.

trasts with the 1938 pattern when the rate of growth tended to rise during August.

INFLUENCE OF TEMPERATURE AND RAINFALL

A practically constant leaf area during the periods discussed above indicated comparable food-making possibilities each week of both years. VERDUIN (15) has demonstrated that for several weeks following the sixth week, approximately, the unit area rate of photosynthesis in maize tends to remain constant. Consequently, an attempt has been made to account for the vari-

ability of weekly increments. Table IV shows the weekly means of temperature based on daily maximum-minimum determinations, the physiological indices of these weekly means according to LEHENBAUER's tables (**7**), and the weekly increments for an 8-week period during each year.

Although LEHENBAUER's indices were determined by measuring elongation of maize seedlings, observations made by the author have shown that dry-weight increases were approximately proportional to elongation rates

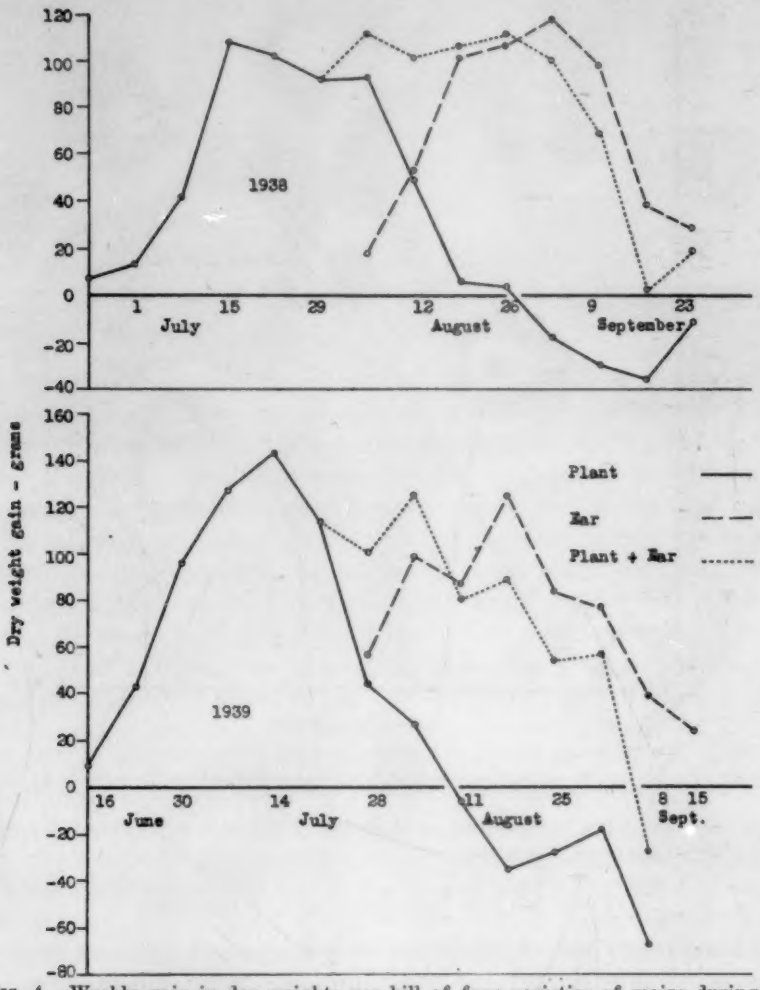

FIG. 4. Weekly gain in dry weights per hill of four varieties of maize during 1938 and 1939. Each point on the curves represents a mean obtained by the harvest of 160 hills.

when moisture and nutrition were optimum. It was, therefore, considered worth while to investigate possible correlations of these indices with dry-

weight increments during the period of growth. A regression study based on the eight weeks' data gave a highly significant coefficient of correlation ($r = 0.81$) between physiological indices and weekly gains for 1939, but the corresponding coefficient ($r = 0.26$) for 1938 was not significant. Records for a ten-week period in 1939 gave a highly significant coefficient of correlation ($r = 0.77$) for calculated temperature indices and dry weight gains,

TABLE IV

WEEKLY MEANS OF TEMPERATURE BASED ON DAILY MAXIMUM-MINIMUM DETERMINATIONS, PHYSIOLOGICAL INDICES OF THESE MEANS, AND WEEKLY MEAN INCREMENTS IN DRY WEIGHT OF 480 MAIZE PLANTS FOR PERIODS OF EIGHT WEEKS IN EACH OF TWO YEARS

WEEK ENDING	WEEKLY MEAN TEMPERATURE	PHYSIOLOGICAL INDICES	INCREMENT IN DRY WEIGHT PER HILL
1938			
	°F.		gm.
July 14	79.6	97	108.0
" 21	75.2	79	102.1
" 28	75.2	79	91.8
Aug. 4	77.6	89	112.7
" 11	78.9	94	101.8
" 18	73.0	69	106.5
" 25	75.6	80	110.8
Sept. 1	71.3	61	117.6*
Mean	75.8	....	.....
1939			
July 6	75.0	78	126.9
" 13	78.4	91	143.7
" 20	72.8	69	112.5
" 27	75.6	81	100.4
Aug. 3	73.7	72	124.7
" 10	68.2	47	86.7
" 17	71.8	64	124.8*
" 24	66.8	41	86.2*
Mean	72.8	....	.....

* Gains in ear weight only.

while the correlation between the actual temperature means and gains ($r = 0.74$) was significant at the five per cent. level only. The relationships for the 8-week periods are presented graphically in figures 5 and 6, together with the amount and distribution of rainfall from the latter part of June until early September.

It is suggested that the failure of the dry weight curve in 1938 to approximate the growth possibilities represented by the temperature index curve may be ascribable to poor distribution of rainfall. In 1938, periods of 18 and 15 days without rain undoubtedly reduced the amounts of water supplied to the plants. Although the rate of photosynthesis in maize tends to be constant whether water levels are high or moderately low (**15**), it has

been shown by LOOMIS (8) that, under conditions of reduced moisture supply, growth of maize is checked and differentiation conditions result in which organic materials are translocated to the roots, and root development is stimulated. In defruiting studies, LOOMIS (9) has demonstrated that the delicate balance in competition between roots and tops for food reserves may be readily disturbed to favor translocation to the roots. Since the data

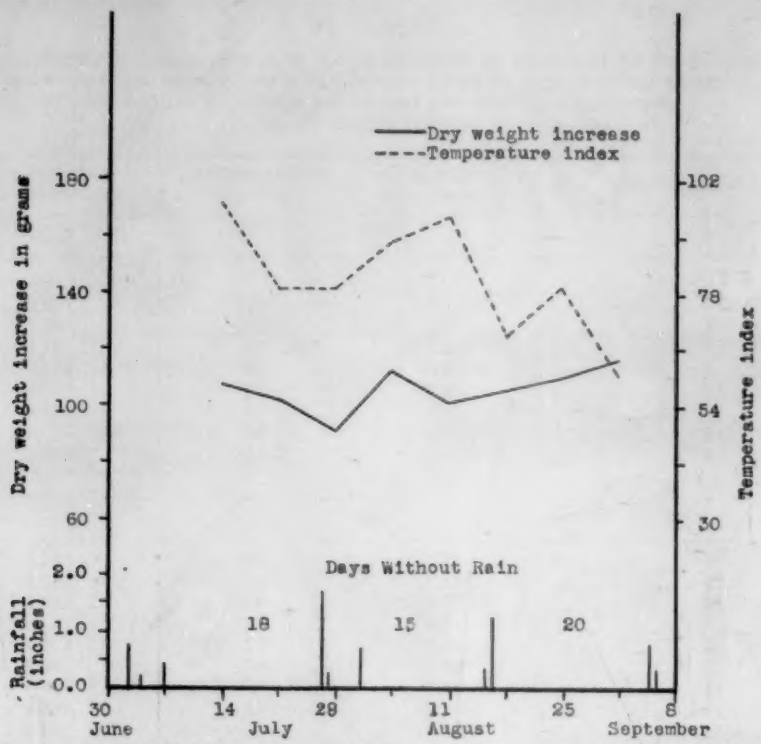

FIG. 5. Physiological temperature indices of weekly mean of daily maximum plus minimum temperatures compared with weekly dry weight increments for 1938.

of table IV show that the eight-week period averaged 3 degrees warmer in 1938 than in the following season, it is probable that, in addition to greater translocation to roots in 1938, a higher rate of respiration further dissipated dry weight reserves.

Unfortunately, sampling of soil moisture (1) during each season was inadequate to permit a quantitative evaluation of its importance as a factor limiting to growth. The difficulty of taking soil samples near the end of a dry period when the end cannot be foreseen is apparent. The moisture data, however, were supplemented by daily tensiometer readings (13) and precipitation records.

In 1939, there was no period of greater than eleven days without rain.

Figure 6 shows that the only week in which the index and the dry weight increment moved far apart climaxed a period of 21 days in which only 0.64 inch of rain had fallen. The fact that moisture was a limiting factor at this stage is substantiated by a rapid rise in tensiometer readings from July 20 to 27. Readings for the instruments at the 36-inch depth averaged 9.0 mm. at the former date, compared with 20.2 mm. a week later. For the 24-inch

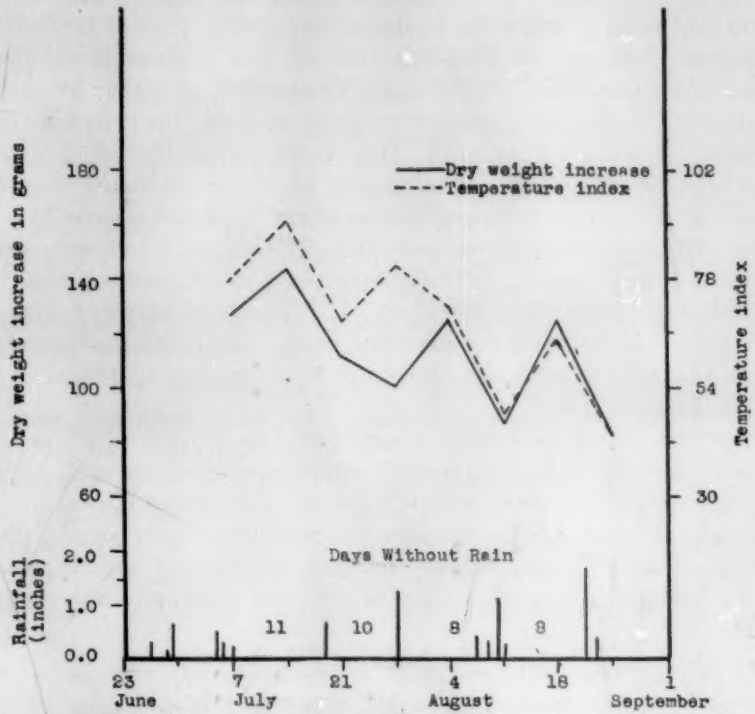

FIG. 6. Physiological temperature indices of weekly mean of daily maximum plus minimum temperatures compared with weekly dry weight increments for 1939.

depth, readings averaged 22.3 mm. on July 20, compared with 37.3 mm. on July 26, after which date the instruments failed to function properly because of faulty connections. Following the rain of July 28 the dry-weight increments resumed their close relationship with the physiological indices.

The 1939 weeks of rapid growth, when the dry-weight curve crossed the comparable 1938 curve, were the weeks ending July 7 and July 14. In addition to favorable physiological indices for these weeks it is noteworthy that this period followed four successive weeks in each of which more than an inch of rain had fallen. The comparable 1938 weeks were those ending July 21 and July 28, and, as shown in figure 5, the dry-weight increments declined in each of these weeks. The rain of July 26 came at the end of an 18-day period without rain during which time tensiometer readings for in-

struments at the 24-inch depth rose from an average of 1.7 mm. on July 7 to 5.2 on July 21, and 26.5 on July 26. The other week in 1938 when the dry weight increment declined from that of the previous week was the week ending August 11. Accompanying this reduction in growth was an increase in the tension of soil moisture as shown by the 24-inch tensiometer readings to 41.9 mm. on August 11, compared with 28.6 mm. on August 4.

The data obtained in these experiments indicate that the growth of corn in the field tends to follow the temperature curve, at least at moderate temperatures. Data not reported here indicate that negative correlations between temperature and growth might be expected at higher average temperatures. In periods of low water supply, however, the expected response to temperature was not obtained. Our figures suggest that soil moisture became limiting for optimum development whenever the plants were forced to draw heavily on the moisture reserves of the third foot of soil (**13**). This critical depth would of course vary with different soil types, and is related to the apparently greater difficulty with which soil moisture is absorbed at increasing distances from the plant (**4**). It should be noted that water deficiencies may reduce the growth rate before they become sufficiently pronounced to cause temporary wilting with its accompanying reduction in the rate of photosynthesis.

Any formula for predicting or explaining the growth of maize on soils of known fertility must integrate the complicated environmental factors of temperature, with its accelerative effect at lower ranges and direct inhibition at higher; it must further consider the moisture supply *in the plant* as affected by rainfall, soil moisture reserves, evaporating environment, and certain internal factors of friction in moisture absorption not yet understood.

Summary

Growth curves, obtained by weekly measurements of the dry weights of four varieties of maize during the growing seasons of 1938 and 1939, showed that the four varieties constituted a single population in their percentage responses to the weekly complex of environmental conditions.

The dry weight curves were sigmoid, accelerating during the first 40 or 50 days, increasing uniformly for the next 50 or 60 days, and dropping off to maturity at 120 to 130 days. In 1939 plantings were made two weeks earlier than in 1938. During the first 50 days after planting the earlier plants developed more slowly; they then showed a sharp rise. Vegetative and total growth for the 1939 (early) plantings were superior for all subsequent points on the curves.

Weekly increments of dry weight, during a period of eight weeks when leaf area was approximately constant, were uniform in 1938 but varied significantly in the cooler, wetter season of 1939. The coefficient of correlation ($r = 0.81$) between weekly gains and LEHENBAUER's physiological indices of

weekly mean temperature was highly significant for 1939 but not significant for 1938. Poor distribution of rainfall apparently accounted for the failure of the dry-weight curve to approximate the growth possibilities represented by the temperature index curve in 1938.

The writer acknowledges the assistance of W. E. Loomis in the preparation of this manuscript.

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GROWTH RESPONSES OF BARLEY SEEDLINGS IN RELATION TO POTASSIUM AND SODIUM NUTRITION

WENDELL R. MULLISON AND ETHEL MULLISON

(WITH THREE FIGURES)

Introduction

In a previous paper (**15**) the effects on young barley plants of variations of the anions and cations in a three-salt nutrient solution were described. The present work was designed to study the effect brought about by various interrelations of the anions of a three-salt nutrient solution in the absence of potassium, using the same general method, and to do further work on the calcium-magnesium relationship which has been reported for other plants (**4**). As will be noted, these original plans were later changed owing to the pronounced growth response of the plants to the substitution of sodium for potassium in the nutrient solution. Work was then done to study the relationship of potassium and sodium in the nutrition of barley plants.

PART I

This phase of the work originally had three separate objectives: A, to determine the optimum amount of potassium in the nutrient solution for barley seedlings; B, to determine the effect of the nitrate, sulphate, and phosphate ions of the nutrient solution upon plant growth in the absence of potassium; and C, to investigate the calcium-magnesium relationship in barley seedlings.

Materials and general methods

Barley seeds selected for uniformity were planted in white, pure quartz sand in glazed self-draining crocks four inches in diameter by eight inches in depth. The seeds were watered with distilled water until they germinated, after which they were at once placed upon the experimental treatments and were given nutrient solution every other day. After germination the seedlings were thinned out to eight plants per pot. A total of about 2000 plants was used in the first runs for these investigations. Several duplicate runs for A and C, which were made in addition, gave similar results. Seedlings were grown under ordinary greenhouse conditions of light and humidity and were harvested when 20 days old. The average height of plants given each treatment was recorded. They were then divided at the sand level into tops and roots, and were dried at 80° C. for 12 hours. Fresh and dry weights of each fraction were determined.

The three objectives mentioned were studied in the following manner:

A.—Eight groups each containing 50 plants were placed in a series

Each was fed with a nutrient solution containing a different amount of potassium. The amounts used were 0, 5, 10, 20, 30, 40, 50, and 60 p.p.m. The solutions were made by substituting in a modified SHIVE's three-salt solution various amounts of NaH_2PO_4 for KH_2PO_4 to give the concentrations of potassium mentioned above. This was done in accordance with the procedure which workers often use when studying potassium deficiency in artificial culture media (**7, 17, 19**).

B.—Three stock solutions, N, S, and P respectively, were used for this triangle in which the anions (nitrates, sulphates, and phosphates) were varied but in which the cations (calcium, sodium, and magnesium) were kept constant. Since it was planned to vary the anions in the absence of potassium, sodium was substituted for that cation throughout. The concentrations of the salts used with the exception of the calcium salts were 4.5 millimolar; the calcium salts were 6.0 millimolar. Solution N was made up of the nitrate salts of sodium, magnesium, and calcium. Solution S consisted of the sulphate salts of sodium, magnesium, and calcium. Solution P contained sodium dihydrogen phosphate, monobasic magnesium phosphate, and monobasic calcium phosphate. Finely powdered calcium sulphate dissolved readily when slowly sifted into solution S. This was easier than the method previously reported (**15**).

Plants at one corner of the triangle were given only the nitrate salts; at a second corner only the sulphate salts; at the third corner only the phosphate salts. All intermediate points were fed with solutions made by mixing together various amounts of N, S, and P so that each point in the triangle varied by $\frac{1}{6}$ from all adjacent points. Fifty plants were set aside for each nutrient treatment in the triangle.

C.—A series of plants was given nutrient solutions which completely lacked potassium and in which all of the anions were kept relatively constant. The calcium and magnesium content was varied by steps of $\frac{1}{6}$ in each solution from the one extreme of all calcium to the other extreme of all magnesium. This was done by mixing together various amounts of solutions M and C. M contained the following magnesium salts, all 4.5 millimolar: nitrate, sulphate, and monobasic phosphate. C contained the nitrate, sulphate, and monobasic phosphate salts of calcium, all 6.0 millimolar.

Control plants were given the modified SHIVE's three-salt nutrient solution composed of the following: 6 millimoles of calcium nitrate; 4.5 millimoles of magnesium sulphate; and 4.5 millimoles of potassium dihydrogen phosphate.

Results

A.—Differences began to appear in 12 to 14 days. At the time of harvest nearly all of the plants grown at the different levels of potassium were in a good vegetative state. Plants grown in the total absence of potassium but

receiving sodium were stunted, and the leaves were yellowed or brown at the tips, while the general color of the plants was a yellowish green. Plants given 5 p.p.m. of potassium were light in color and had a few yellowed leaf tips. Those given 10 p.p.m. were not quite as vigorous as those grown at higher levels but the differences in their weights were not marked. The data are given in table I.

TABLE I

DRY WEIGHTS (IN GRAMS) OF GROUPS OF 50 PLANTS GROWN AT DIFFERENT LEVELS OF K. VARIOUS AMOUNTS OF NaH_2PO_4 SUBSTITUTED FOR KH_2PO_4 IN A COMPLETE SOLUTION TO GIVE THE REQUIRED P.P.M. OF K

	P.P.M. OF K								
	0	5	10	20	30	40	50	60	180 (CONTROL)
Tops	1.22	1.44	1.53	1.57	1.58	1.62	1.84	1.39	1.75
Roots	1.29	1.17	1.33	1.50	1.59	1.59	1.61	1.50	1.53
Total	2.51	2.61	2.86	3.07	3.17	3.21	3.45	2.89	3.28

B.—Here the effects brought about by the variation of the anions in the nutrient solutions where potassium was replaced by sodium were not very different from those effects, already reported (**15**), brought about in the presence of potassium. Nevertheless, at the time of harvest the plants were a lighter green than normal, this undoubtedly being the first sign of potassium deficiency. Among those plants on a low nitrogen level this yellow color was more pronounced owing to nitrogen starvation as well as to lack of potassium. These latter plants were stunted as well as yellowed. Data showing the dry weights and heights are given in figure 1. Data for the control of B are given in the legend of figure 1.

C.—Deficiency symptoms appeared here astonishingly quickly. Within three or four days after the first application of the deficient solutions the older leaves became yellowed at the tips and thereafter died back rapidly (**3**). At the time of harvest these symptoms were severe and if the plants had been left longer before harvesting they would undoubtedly have died completely. It apparently did not matter whether calcium was high and magnesium low or vice versa; in either case the plants died back with equal rapidity presumably owing to the lack of potassium. Data concerning the dry weights are given in table II. Control plants were vigorous and showed no deficiency symptoms.

When the results of A, B, and C are compared, several points are seen to be salient. First, the potassium-deficient plants in C were affected a week to ten days before the plants in A and B, and the deficiency symptoms were much more pronounced and severe. Secondly, in B no new effects were

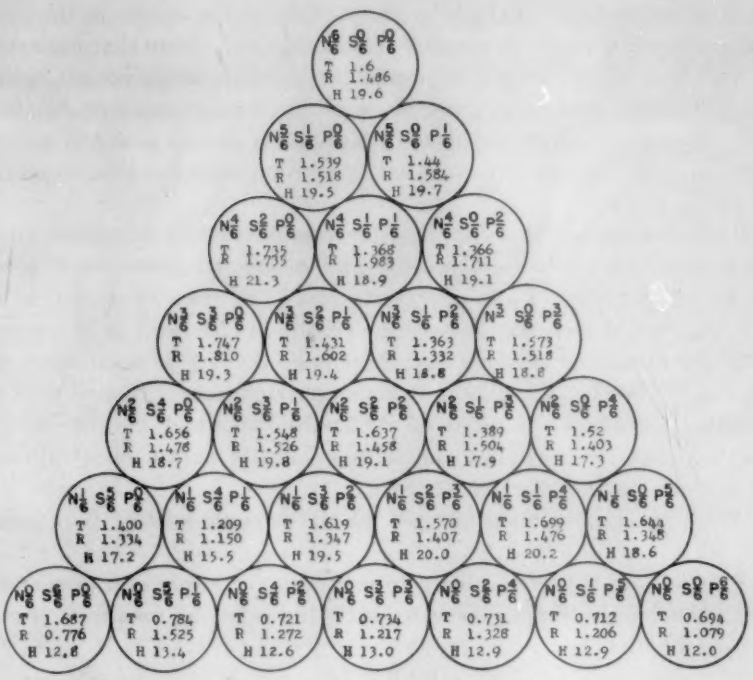

FIG. 1. **Results of part I, B. Each point of triangle, representing data of 50 plants, was watered with the fractions of solutions N, S, and P indicated in each circle. Solution N contained various nitrates; S, sulphates; and P, phosphates. Numbers refer to dry weights in grams of tops and roots, and average heights in cm. Data for control: tops, 2.535 gm.; roots, 1.919 gm.; height, 20.5 cm.**

TABLE II

DRY WEIGHTS (IN GRAMS) AND AVERAGE HEIGHTS (IN CM.) OF GROUPS OF 25 PLANTS GIVEN SOLUTIONS CONTAINING VARIOUS AMOUNTS OF Mg AND Ca BUT NO K OR Na

	FRACTIONS OF STOCKS "M" AND "C" MAKING UP EACH SOLUTION							
	M1, C0	M⅚, C⅙	M⅔, C⅓	M½, C½	M⅓, C⅔	M⅙, C⅚	M0, C1	COMPLETE SOLUTION
Top weight	0.31	0.57	0.49	0.46	0.42	0.28	0.21	1.02
Root weight	0.63	0.67	0.76	0.63	1.07	0.86	0.86	1.58
Total weight	0.94	1.24	1.25	1.09	1.49	1.14	1.07	2.59
Average height	9.4	15.4	19.0	15.7	13.6	10.0	9.4	18.6

brought about by varying the relative amounts of the anions in the absence of potassium other than those found in its presence. Note that here sodium salts had been substituted for potassium salts in the nutrient solution. Third, there was general agreement as to the time of occurrence and the severity of the potassium deficiency symptoms in A and B at the zero level of potassium, but results of C were not at all comparable in this respect with results of A and B.

With this in mind, later experiments were devised to determine answers to these questions: 1. Were the swift and severe symptoms in C brought about by the toxicity of the phosphate ions? If they were due to some toxicity this might be likely because the results in C as well as in a previous preliminary investigation show that there is no toxicity of magnesium when calcium is at a low level or absent at the magnesium concentration used here. Other investigations have indicated that phosphates may be toxic and that sulphates and nitrates are not, unless in unusually high concentrations (**5, 14, 15**), which was not the present case.

2. Were these symptoms brought about through lack of potassium in itself?

3. Can sodium actually substitute partially for potassium during early growth of barley seedlings as is apparently indicated by results of A and B as compared with C?

PART II

A.—The solutions used in "C" of part I had contained twice the amount of phosphate which was present in the controls. To determine whether phosphate toxicity was causing the severe die-back, and if pH might be linked with it, as well as to determine whether Na had been substituting for K, each of the following solutions was fed to a different group of young seedlings, each group containing 50 plants. The experiment was later repeated.

1. Control solution, a modified SHIVE's three-salt solution.

2. Solution 1 acidified to pH 3.8 through the addition of H_2SO_4. In all cases where low pH was involved the nutrient solution was adjusted to the desired pH and plants were watered with it. The pots were not flushed with acidified water in between.

3. Solution 1 acidified to pH 2.2 through the addition of H_2SO_4.

4. −K and −Na; otherwise same ions present as in 1.

5. −K and −Na; twice as much phosphate present as in 1.

6. −K and −Na; phosphate concentration half that of 1.

7. −K and −Na; corresponds to solution made by mixing $\frac{1}{2}$M + $\frac{1}{2}$C used in "C" of part I, but modified so that the NO_3 concentration was increased by half, and the PO_4 concentration decreased by half.

8. Control solution, but with NaH_2PO_4 substituted for KH_2PO_4.

9. −K, +Na; twice as much PO_4 and Na as 8.

10. $-K$ and $-Na$; corresponds to solution made by mixing $\frac{5}{6}M + \frac{1}{6}C$ used in "C" of part I.

11. Same as 10 except that proportion is $\frac{1}{6}M$ to $\frac{5}{6}C$.

12. $-K, +Na$; same as $\frac{1}{2}M + \frac{1}{2}C$ but with 4.5 millimoles of Na_2SO_4 added.

The pH and compositions of these solutions are given in table III.

TABLE III

PH AND MILLIMOLE COMPOSITIONS OF THE 12 CULTURE SOLUTIONS

	1, 2, 3*	4	5	6	7	8	9	10	11	12
$Ca(NO_3)_2$	6.0	6.0	6.0	6.0	4.5	6.0	6.0	1.0	5.0	3.0
$MgSO_4$	4.5	2.2		3.4	2.3	4.5	4.5	3.7	0.75	2.3
KH_2PO_4	4.5									
H_2SO_4		2.2	4.5	1.1						
$Mg(H_2PO_4)_2$		2.2	4.5	1.1	1.1			3.7	0.75	2.3
$Mg(NO_3)_2$					3.4			3.7	0.75	2.3
$CaSO_4$					3.0			1.0	5.0	3.0
$Ca(H_2PO_4)_2$					1.5			1.0	5.0	3.0
NaH_2PO_4						4.5	9.0			
Na_2SO_4										4.5
pH	1 – 4.8 2 – 3.8 3 – 2.2	2.6	2.2	3.0	2.8	4.8	4.2	4.6	3.8	4.0

* pH varied by addition of H_2SO_4.

Results of applying these various solutions to the sand cultures are shown in table IV, which gives data for the dry weights and heights of the plants.

TABLE IV

DRY WEIGHTS (IN GRAMS) OF GROUPS OF 50 PLANTS

	SOLUTION NUMBER											
	1	2	3	4	5	6	7	8	9	10	11	12
Tops	2.36	2.35	1.10	1.15	0.66	0.80	1.00	1.65	1.53	1.13	0.77	1.53
Roots	1.05	1.10	0.91	0.78	0.68	0.90	0.87	0.99	1.01	0.76	1.02	0.88
Total	3.41	3.45	2.01	1.93	1.34	1.70	1.87	2.64	2.54	1.89	1.79	2.41

In omitting the Na and K ions from certain of the solutions, all or part of the SO_4 was added as H_2SO_4; by this means no additional new ion would have to be introduced and the concentration of all the ions could be kept approximately the same as in the control solution 1, so that accurate comparisons could be made. This was true of solutions 4, 5, and 6. In each case this brought the pH down to between 2.2 and 3.0. Solution 3, which was solution 1 acidified to pH 2.2, was used as a check, and it can be seen that the low pH did have a detrimental effect upon the dry weight as compared with plants given solution 1. Nevertheless, when noting results of

solution 4 as compared with solutions 5, 6, and 7, all lacking both Na and K, and all of very low pH, it can be seen that varying the concentration of PO_4 as was done here makes no significant difference in the final dry weights at that low pH, although there may be some PO_4 toxicity indicated in 5. Since there are only the slightest indications of PO_4 toxicity under such unfavorable conditions as these pH values it would seem that under more favorable conditions for growth the PO_4 would not prove to be toxic at all. That this was so at the concentrations used in part I, C, can be seen by comparing results of 8 and 9; these solutions are essentially similar except for the PO_4 concentration. Since the solutions used in part I, C, were all above pH 4.0, it is therefore clear that the severe dieback could not have been caused by the concentration of the PO_4 or by the pH, but must have been caused by the fact that there was no Na present to substitute for the lack of K.

Since it is not possible to make a solution with the same ionic concentrations, as 1 and 8, and which will lack both Na and K without lowering the pH considerably, the solutions used instead were 10, and 11, which thus served as an additional check on the earlier work. Although the former is high in Mg and the latter high in Ca, the dry weights produced by each when applied to the sand cultures were almost the same, and were less than the dry weights produced by 8, which has Na added, and much less than 1, which has K instead of Na. A further comparison of results of 10 and 11 with those of 12 show that when Na_2SO_4 is added to a solution like that used in C, the results are brought into conformity with results of A and B. This is further brought out by comparing results of 12 with 10, 8, and 1. Therefore in A, and B, sodium must have substituted at least partially for potassium since the symptoms there were so much less severe.

B.—The object of this experiment was to study the interrelations of Na and K and to determine approximately to what extent the two are interchangeable. For each run about 1500 barley seeds were planted in two-gallon, self-draining glazed crocks in pure quartz sand and were watered from the time of germination with various nutrient solutions. Each crock was thinned to contain 25 plants. They were divided into two groups. Crocks of one group were given solutions containing the following p.p.m. of K, respectively: 0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 100, 140, and 180. The other group in addition had enough sodium added to the solution given each crock so that the total concentration of sodium plus potassium ions given to any one was 180 p.p.m. which is approximately the amount of K in the control nutrient solution used here. Thus half the plants were receiving only varying amounts of K while the other half were receiving varying amounts of K + Na. In addition to the amounts of K and K + Na just noted, one group of plants was given 180 p.p.m. of K + 90 p.p.m. of Na; another was given 180 p.p.m. of K + 180 p.p.m. of Na. Groups of fifty plants were given

each type of nutrient solution. The basic – K solution consisted of 6.0 millimoles of calcium nitrate, 2.3 millimoles of magnesium sulphate, and 2.3 millimoles of magnesium phosphate, and had a pH of 5.1. K was added as KCl and Na was added as NaCl.

To act as a check upon the chloride ion, two groups of plants were set aside. One group was given sodium chloride, and the other group was given sodium sulphate. Results showed that the chloride ion had no detectable effect upon the dry weight or heights of plants grown under these conditions.

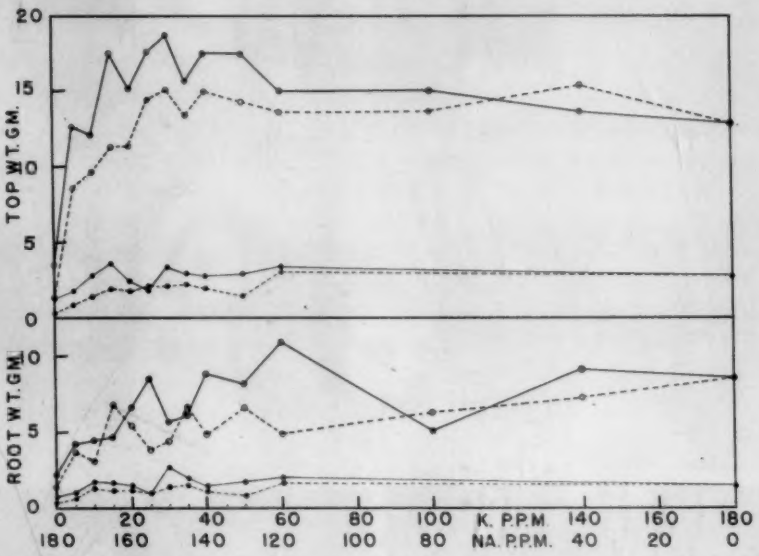

FIG. 2. Results of part II, B. Dry weights in grams of tops and roots, each point representing data for 50 plants. Dotted lines: plants given K but no Na. Solid lines: plants given total of 180 p.p.m. K + Na; *e.g.*, the fifth circle of upper solid line represents plants having been fed 20 p.p.m. of K + 160 p.p.m. of Na; corresponding point on dotted line shows plants that had 20 p.p.m. of K and no Na. Upper graph in each case is of tops and of roots 70 days old; lower graphs of plants 40 days old.

During the course of the work all the crocks were shifted about so as to minimize local differences of environmental factors in the greenhouse. The experiment was repeated twice, the last time with older plants. The first set was harvested when 40 days old; the second run gave confirmatory results which are therefore not given here. The third set were allowed to grow on until 70 days old in order to determine whether the same results would be obtainable beyond the seedling stage. Data are given for an older and a younger set in figure 2. This figure, however, does not include results obtained when plants were given 180 p.p.m. of K + 90 and 180 p.p.m. of Na, respectively. In certain cases the additional Na brought about a decided

increase in the dry weight even when 180 p.p.m. of K was already present. One such case in shown in figure 3, D.

The plants given no Na showed a gradual increase in height, color, and vigor from 0 up to 30 to 40 p.p.m. of K, which was apparently the optimum

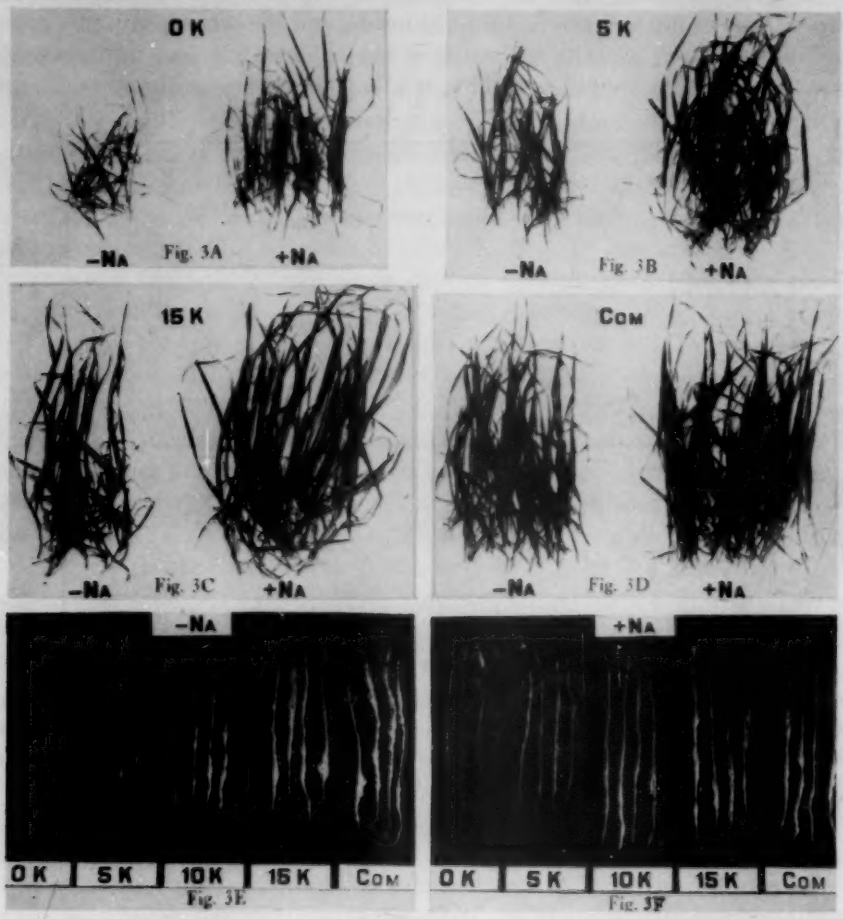

FIG. 3. Photographs of tops and roots of seedlings given varying p.p.m. of K with and without Na in an otherwise complete nutrient solution. The complete solution contained 180 p.p.m. of K and no Na. The + Na plants of fig. 3D had Na added to the complete solution.

amount. Plants given more than that were about the same in appearance and dry weight. Plants given Na in addition to the K did not show the pronounced gradation over as long a range as did those given only K. The former were superior in every respect, though the most pronounced improve-

ments were found only up to about 60 p.p.m. and were most marked at the lower K levels. The differences between the plants given no Na and no K and those given 180 p.p.m. of Na and no K were especially marked. The former made little growth even in early stages, then began to die back rapidly; at the time of harvest, in one run, only 16 of the original 50 plants were alive; of the plants given the Na, 39 were alive and although they were not vigorous yet several of them had begun to stool. It was found that in several cases even the plants given 180 p.p.m. of K were definitely stimulated by the addition of Na.

Discussion

The idea that sodium can be partially substituted for potassium is not a new one. Some early work on this problem was done as far back as 1860. Probably the best-known early work was that of HELLRIEGEL in 1898 as mentioned in RUSSELL'S text (**18**). Some of the earliest work in this country was done at the Rhode Island Agricultural Experiment Station (**8, 9**). Then for a long time not much attention was paid to this problem. Within the last few years, however, experiments have again been carried on with certain field crops, with results indicating that sodium is quite important under certain conditions of plant nutrition. This is particularly true of sugar beets and cotton (**12, 13**). LEHR (**10, 11**) even states that for beets sodium may almost be deemed an indispensable nutrient element, approaching potassium in importance. Very favorable increases in yield have been obtained with other crops as well (**2, 6**). A rather comprehensive review of European literature in this field is given by LEHR (**10**).

Certain workers have found that sodium will produce increased yields when the potassium supply is low. BUTKEVICH and MARUASHVILI (**1**) have stated that sodium can be substituted for as much as 25 per cent. to 75 per cent. of the potassium without detrimental results in yield. In fact, they state that at periods of stalk formation and heading in wheat the complete elimination of K and its replacement with Na in the culture solution results in a marked increase in yield. Still others have found that sodium salts give increases in crop yield in the field even when potassium salts are present (**2, 18**). Of course, different plants vary in their responses to this element. For example, HARTT (**7**) concluded from her experimental work that sodium could not be substituted for potassium in sugar cane.

But even though this is no new idea it has been almost neglected in work which has been done by the plant physiologist using artificial culture media. Most workers have assumed that sodium cannot replace potassium at all. The fact that plants could grow perfectly well in a culture medium entirely lacking in sodium doubtless caused the assumption that the plants would not utilize it. Work carried on simultaneously with the present work, however, and published elsewhere (**16**) indicates that sodium can be substituted to

some extent for potassium in young tomato plants grown under controlled nutrient conditions, and the present work indicates the same for both barley seedlings and plants grown through a period of 70 days. To what extent this affects the chemical composition of the plants in question is not known but the presence of sodium certainly affects the speed as well as their severity, with which the potassium deficiency symptoms appear. It also affects the amount of dry matter synthesized by the plant. It has been shown here that potassium deficiency is one of the first and severest of any of the mineral deficiencies to manifest itself, killing the plants to the ground rapidly in the young stage. But barley plants given sodium in place of potassium are even enabled to make a small amount of growth before showing potassium deficiency symptoms. These plants remain alive for a long period of time and are larger and synthesize more dry matter than those lacking both potassium and sodium; the latter die quickly. It is therefore probable that the sodium itself is utilized in place of the potassium when the latter is present in insufficient amounts. Therefore, to get an accurate picture of the effect of potassium on plants, sodium as well should be excluded from the nutrient solution.

Conclusion and summary

1. The effects brought about by the variation of the anions in a nutrient solution where potassium was replaced by sodium were not very different from those effects, already reported, brought about in the presence of potassium.

2. The severe dieback reported here of plants grown without either Na or K was found to be due to a deficiency of K rather than to interrelations of PO_4 and NO_3, or Ca and Mg, or a toxicity of one of these.

3. About 30 to 40 p.p.m. of K is optimum for growth of young barley seedlings under the conditions of these experiments.

4. In the absence of both Na and K, K deficiency symptoms appear much earlier and are much more severe than when Na is present. In fact, this is one of the earliest of any of the deficiencies to appear in barley.

5. In young stages of growth Na can and does replace K to some extent even though it cannot replace it entirely. This is especially marked when K is low in amount.

6. When studying the effect of K at various nutritional levels sodium must be excluded from the nutrient solution in order to obtain an accurate picture of the rôle of K in plant metabolism.

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COMPARISON OF THE EFFICIENCY OF SINGLE APPLICATIONS WITH REPEATED TOP DRESSINGS OF NITROGENOUS FERTILIZERS IN INCREASING THE YIELD OF DRY MATTER, NITROGEN, AND VITAMIN C (ASCORBIC ACID) OF SUDAN GRASS[1]

F. Lyle Wynd

Introduction

The rapid response of grasses to top dressings of nitrogenous fertilizers is well known. Many types of grass crops such as permanent pastures, golf courses, and lawns obviously may not be fertilized once they have become established except by surface applications. This practice is also frequently followed in the culture of other crops. The general belief is that a light top dressing with nitrogenous fertilizer shortly before harvest induces a rapid increase in the protein content of the crop. There is considerable evidence that this sudden increase in the absorption of inorganic nitrogen may not always increase the protein content significantly because of disturbed synthetic processes which may accompany it. This problem, however, lies beyond the scope of the present study.

The purpose of the present study was to investigate the comparative efficiencies of nitrogenous fertilizers added in a single application at the time of seeding and when only a part was added at this time with the remaining part added as a top dressing at a later period. This problem seems especially worthy of investigation in view of the increasing production of artificially dried immature grasses in this country. The rapid growth following the repeated cuttings of these crops exerts a heavy drain on the available nitrogen in the soil, and hence nitrogenous fertilizer usually must be added during the season to maintain the high nutritional value of the crop. The increasing difficulty of obtaining adequate supplies of nitrogenous fertilizers especially emphasizes the necessity of their use under conditions of maximum efficiency.

Methods

The experiments were carried out on a field of typical Brennan Fine Sandy Loam near Elsa, Texas, which was selected on the basis of its previous history of a high and uniform productivity of Sudan grass. The field was plowed eight inches deep July 3, 1941. It was then single-disked, harrowed, and levelled. The experimental plots were each one-hundredth of an acre in size. Control plots were arranged adjacent to each treated plot. The

[1] This study was carried out with funds granted to the Graduate School of the University of Illinois by the Cerophyl Laboratories, Inc., Kansas City, Missouri.

calculated amounts of ammonium sulphate and ammonium phosphate were added by hand July 10. The field was then double-disked and immediately seeded to Sudan grass. One series of plots received at this time the entire amount of fertilizer at the rates indicated in the table. Another series received half of the amount at this time and the remaining half was added as a top dressing immediately after the first cutting. Immediately following the second application of fertilizer all plots were irrigated by an overhead sprinkler system which leached the fertilizer into the upper few inches of soil.

The efficiency of the fertilizer was calculated as the percentage increase in the yield of dry matter, nitrogen, and vitamin C per acre per "unit" of nitrogen added in the fertilizer. These yields are the sums of the first and second cuttings. The percentage increase in these yields was calculated in respect to the yields of the individual adjacent control plots. For this reason, the yields themselves are not directly comparable.

A "unit" of nitrogenous fertilizer is considered to be 100 pounds of ammonium sulphate per acre or an equivalent amount of nitrogen as ammonium phosphate. On this basis, 400 pounds of ammonium sulphate and 600 pounds of ammonium phosphate would each represent 4 "units" of nitrogen fertilizer per acre, and 500 pounds of ammonium sulphate and 750 pounds of ammonium phosphate would each represent 5 "units" of nitrogen. These equivalencies are based on the chemical analysis of the particular commercial brands of fertilizer used.

The first cutting was made August 3, 25 days after seeding. The second cutting was made August 14, 36 days after seeding, or 11 days after the first cutting. The harvested material was quickly dried at 180° F. in a forced draft dehydrator.

Vitamin C was determined in fresh material as follows, and the results calculated in terms of yields in grams per acre: An aliquot of 25 milliliters of an acid extracting solution was added to 2 grams of fresh grass and the mixture then thoroughly ground in a mortar with 5 grams of pure Ottawa sand. The extracting solution contained 2 per cent. metaphosphoric acid and 3 per cent. trichloracetic acid. The triturated sample was filtered through no. 202 Reeve Angel filter paper, and 3 milliliters of the filtrate was titrated with a standard dye solution. All titrations were made in triplicate and the average values were used as the basis for calculating the yields per acre. The standard dye solution was prepared by dissolving 55 mg. of sodium 2,6-dichlorobenzenoneindophenol in 40 ml. of hot water in a 50-ml. volumetric flask. After cooling to room temperature, the solution was made up to 50 ml., filtered, and standardized by titration against a known amount of ascorbic acid. The standard solution of ascorbic acid was

made up by dissolving 10 mg. of ascorbic acid in 50 ml. of the acid extracting solution.

The total nitrogen content in the Sudan grass was determined by the Kjeldahl procedure modified to include nitrate nitrogen. One gram of the dried sample was placed in an 800-ml. Kjeldahl digestion flask and treated with 30 ml. of a digestion mixture. This digestion mixture was prepared by dissolving 15 gm. of copper sulphate in a small amount of hot water and adding this solution and 125 gm. of salicylic acid to 2.5 liters of sulphuric acid. After standing for 1 hour in the digestion mixture, the flask was heated for 5 minutes and then 5 gm. of sodium thiosulphate were added. After rotating the flasks on the burners for a few minutes, 10 to 15 gm. of powdered potassium sulphate were added and the mixture digested for 2 hours. The digested sample was cooled, diluted to 250 ml., 100 ml. of 50 per cent. sodium hydroxide added and distilled into 4 per cent. boric acid. The distillate was titrated with 0.01714 N hydrochloric acid.

Results

The data concerning yield in dry matter are presented in table I. It is apparent that there is a considerable increase in the efficiency of both ammonium sulphate and ammonium phosphate when the entire indicated amounts were added at the seeding time. For the ammonium sulphate, there was an 8 per cent. increase in the efficiency at the 400 pounds per acre level when the entire amount was added at seeding time. At the 600 pounds per acre level, this increase in efficiency was 14 per cent.

TABLE I

TOTAL YIELD IN DRY MATTER IN THE FIRST 2 CUTTINGS OF SUDAN GRASS. COMPARISON OF SINGLE WITH DOUBLE APPLICATIONS OF FERTILIZER

DATA	AMMONIUM SULPHATE				AMMONIUM PHOSPHATE			
	400 LB. PER ACRE		600 LB. PER ACRE		500 LB. PER ACRE		750 LB. PER ACRE	
	1 APPL.	2 APPL.	1 APPL.	2 APPL.	1 APPL.	2 APPL.	1 APPL.	2 APPL.
Control plots, yield, lb. per acre	1470	1745	1470	1745	1470	1745	1470	1745
Fertilized plots, yield, lb. per acre	2255	2595	2300	2615	2182	2250	2530	2665
Pounds per acre increase due to fertilizer	785	850	830	870	712	505	1060	920
Percentage increase due to fertilizer	53	49	57	50	49	29	72	53
Percentage increase per nitrogen unit of added fertilizer	13.2	12.2	9.5	8.3	12.2	7.2	12.0	8.8
Percentage increase in efficiency	8	.....	14	.....	70	.....	37	.....

The ammonium phosphate applications present even more striking results. At the 500 pounds per acre level, there was a 70 per cent. increase in efficiency when the entire amount was added at seeding time. At the level of 750 pounds per acre, the increase in efficiency was 37 per cent. It is evident that ammonium phosphate is much superior to ammonium sulphate, at equivalent nitrogen levels, in increasing the yield of Sudan grass under the conditions of the experiment. This is especially true at the lower levels of application.

The data concerning the recovery of nitrogen by the crop are presented in table II. The results are roughly similar to those obtained for the yield

TABLE II

TOTAL NITROGEN RECOVERED IN THE FIRST 2 CUTTINGS OF SUDAN GRASS. COMPARISON OF SINGLE WITH DOUBLE APPLICATIONS OF FERTILIZER

DATA	AMMONIUM SULPHATE				AMMONIUM PHOSPHATE			
	400 LB. PER ACRE		600 LB. PER ACRE		500 LB. PER ACRE		750 LB. PER ACRE	
	1 APPL.	2 APPL.	1 APPL.	2 APPL.	1 APPL.	2 APPL.	1 APPL.	2 APPL.
Control plots, N recovery in lb. per acre	44.5	43.9	44.5	43.9	44.5	43.9	44.5	43.9
Fertilized plots, N recovery in lb. per acre	79.4	75.6	82.6	79.7	66.2	57.5	81.8	76.1
Lb. per acre increase due to fertilizer	34.9	31.7	38.1	35.8	21.7	13.6	37.3	32.2
Percentage increase due to fertilizer	76	69	86	79	49	29	84	71
Percentage increase per nitrogen unit of added fertilizer	19.0	17.2	14.3	12.2	12.2	7.2	14.0	11.8
Percentage increase in efficiency	10	.....	15	.....	70	.....	19	.....

in total dry matter. In each instance there is an increased efficiency in the total nitrogen recovered by the crop when the entire amount of nitrogenous fertilizer was added at seeding time. When 400 pounds of ammonium sulphate were added per acre, this increased efficiency was 10 per cent. At the 600 pounds per acre level, this figure increased to 15 per cent.

The ammonium phosphate applications yield efficiency values of greater magnitude. At the 500 pounds per acre level, there was an increase of 70 per cent. in the efficiency per nitrogen unit of fertilizer added to the soil when the total amount was added at seeding time. At the 750 pounds per acre level, the increased efficiency was 19 per cent.

Just as in the case of total yields of dry matter, ammonium phosphate appears to be superior to ammonium sulphate, at equivalent levels of nitrogen, in increasing the amount of nitrogen recovered by Sudan grass under

the conditions of the present experiment. This appears to a remarkable degree at the lower levels of fertilizer application.

The yields in vitamin C are presented in table III. An examination of

TABLE III

TOTAL YIELD OF VITAMIN C IN THE FIRST 2 CUTTINGS OF SUDAN GRASS. COMPARISON OF SINGLE WITH DOUBLE APPLICATIONS OF FERTILIZER

DATA	AMMONIUM SULPHATE				AMMONIUM PHOSPHATE			
	400 LB. PER ACRE		600 LB. PER ACRE		500 LB. PER ACRE		750 LB. PER ACRE	
	1 APPL.	2 APPL.	1 APPL.	2 APPL.	1 APPL.	2 APPL.	1 APPL.	2 APPL.
Control plots, vitamin C yield in grams per acre	1649	1487	1649	1487	1649	1487	1649	1487
Fertilized plots, vitamin C yield in grams per acre	2420	3010	2270	3000	2060	1855	2230	2700
Grams per acre increase due to fertilizer	771	1523	621	1513	411	368	581	1213
Percentage increase due to fertilizer	147	203	138	202	125	125	135	182
Percentage increase per nitrogen unit of added fertilizer	37	51	23	34	31	31	23	31
Percentage increase in efficiency	.....	35	.....	50	.....	0	.....	35

these data shows that the greatest efficiency per unit of nitrogenous fertilizer applied is obtained when the total amount is added in 2 applications rather than as a single initial treatment. This is directly opposite to the results of the yield of total dry matter and the recovery of total nitrogen by the crop. When ammonium sulphate was added at the rate of 400 pounds per acre, there was an increase of 35 per cent. in the efficiency of the fertilizer per unit of added nitrogen when one half of the total amount was added at the time of seeding and the remaining half added as a top dressing immediately following the first cutting. At the level of 600 pounds per acre of fertilizer, this efficiency had increased to 50 per cent. The greater efficiency thus appears to exist at the higher rates of application.

The efficiency of ammonium phosphate is of smaller magnitude than that of ammonium sulphate. In fact, at the lower rate of application, no change in efficiency was noted in the yield of vitamin C per acre. At the higher rate of application, however, an increase of 35 per cent. in fertilizer efficiency was noted.

Discussion

The general belief that top dressing is the most economical method of applying nitrogenous fertilizer is based on 2 considerations. In the first place, the shallow root system of many species of grass presumably would

have an opportunity to come into more immediate contact with the added nutrients; and second, that loss of nitrogen from the soil by leaching is reduced to a minimum. If the grass which is being studied actually is a shallow-rooted species, and if there is real danger of nitrogen loss from the soil, then frequent, light top dressings of fertilizer would probably be the most efficient method of its application.

Many agriculturally important plants, however, possess root systems of much greater depth than is commonly supposed. For example, Sudan grass 25 days after planting, under the conditions of the present experiment was about 20 inches tall when measured from the surface of the soil to the average extreme extension of the leaves. The plants had not yet developed joints. Bunches of the plants could be easily pulled up by hand and such superficial examination as this made possible indicated that most of the roots were confined in the upper few inches of soil. Yet careful dissection of the soil profile showed that a considerable quantity of young, feeding roots had penetrated to a depth of 3.5 feet! This evidence alone indicates that fertilizer added as a top dressing does not necessarily come into immediate contact with the majority of the feeding roots.

The loss of soluble nitrogen from the soil by leaching presumes that the rain water and irrigation water percolates to a depth beyond the effective feeding range of the roots. In regions of humid climates this percolating water would reach the water table and hence such substances as were in solution at this depth would be lost permanently from the soil profile. This is one of the important characteristics of the podsolic type of soils. On the other hand, in the more arid regions in which the typical pedocal soils develop, only rarely does surface water reach the water table. The average depth of penetration of rain water in the climatic area which includes the Brennan soil series is from 3 to 5 feet, depending on the texture and structural variations in the soil profile. This indicates that leaching cannot remove soluble nitrogen from the feeding zone of the roots of Sudan grass when grown on soils of this series.

The deep root system of a "tall grass" species such as Sudan grass and the limited depth of soil leaching furnishes a basis for interpreting the greater efficiency of nitrogenous fertilizer in increasing the yield of dry matter and also the recovery of nitrogen by the crop when the entire amount is applied at the time of seeding. When the entire amount is added at seeding time, the combined action of rainfall and irrigation tends to move the fertilizer downward and distribute it throughout the soil profile. It then comes in contact with the expanding root system in the lower zones of nutrient absorption. On the other hand, when a smaller application is made at the time of seeding, the added nitrogen is largely absorbed by the first crop. The fertilizer added as a top dressing after the first cutting, and

only 11 days before the subsequent cutting, is necessarily limited to the upper few inches of soil and it does not have time to become distributed throughout the profile through which the feeding roots are themselves already distributed.

Further studies are in progress to determine the efficiency of nitrogenous fertilizer when added at various periods in the life history of the plant and the relation of their efficiency to the distribution of the fertilizer in the soil profile.

Summary

1. Sudan grass was grown on Brennan Fine Sandy Loam. The first cutting was made 25 days after planting. A second cutting was made 11 days later.

2. The relative efficiencies of ammonium sulphate and of ammonium phosphate were studied when a given amount was added at the time of seeding and when part was added at seeding and the remainder as a later top dressing.

3. The greater efficiency per unit of added nitrogen in increasing the total yield of dry matter was found when the entire amount of fertilizer was added at seeding time. Ammonium phosphate was significantly more efficient per unit of added nitrogen than was ammonium sulphate.

4. The greater efficiency per unit of added nitrogen in increasing the recovery of nitrogen by the 2 cuttings also was obtained when the entire amount of fertilizer was added at seeding time. Ammonium phosphate was more efficient per unit of nitrogen added than was ammonium sulphate.

5. The total yield of vitamin C per acre varied inversely as the yield of dry matter and the recovery of nitrogen by the crop. The greater efficiency per unit of nitrogen added was found when part of the fertilizer was added at the time of seeding and part just following the first cutting. Ammonium sulphate was more efficient per unit of nitrogen than ammonium phosphate.

6. A tentative explanation is offered of the comparative efficiency of nitrogenous fertilizer based on its distribution throughout the soil profile and on the depth of the root system of the plant.

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THE AMOUNT OF BORON ABSORBED BY SOYBEAN PLANTS AND ITS EFFECT ON THEIR GROWTH[1]

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(WITH ONE FIGURE)

Introduction

There is a large literature in regard to the effects of boron toxicity and deficiency in plants. A recent review has been given by McMurtrey (1). These papers have been concerned primarily with the external symptoms of deficiency and toxicity in the plant. More recently, analytical methods have been perfected so that boron analyses may be conducted in a routine manner.

Plant symptoms have indicated that many areas of soil do not contain a sufficient supply of available boron for the normal growth of some vegetable and hay crops. A search of the literature reveals very little actual information as to the relation of the boron content of soils or of soil factors which may determine the availability of boron to the plant.

Experimental procedure

The use of a mixture of sand and soil as a culture medium for growth studies on the soybean plant was made to ascertain whether growth responses to boron treatment could be correlated to foliar symptoms and the boron content of the plant. A short term growth period as well as the mixture of sand and soil as a culture medium was utilized in order that small samples of soil, available for other laboratory uses, might be tested for boron toxicities under greenhouse conditions.

A high-grade acid-washed quartz sand was mixed in equal proportions, on a dry basis, with a Maury silt loam soil. Small aluminum culture vessels, each containing 100 grams of the dry medium were used for individual planting. No additions of nutrient were made to the sand-soil medium, other than traces of copper, manganese, and zinc which were added to the distilled water used to maintain the moisture content of the cultures at a level of 60 per cent. saturation.

Purified sand cultures in similar culture vessels were prepared as a comparison to treatments in the sand-soil medium. These were saturated before planting and watered thereafter with a nutrient solution consisting of:

[1] Contribution from the Department of Chemistry, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

167.4 ml. of 0.5 M $MgSO_4$
167.4 ml. of 0.5 M KH_2PO_4
208.9 ml. of 0.5 M $Ca(NO_3)_2$
18.2 ml. iron citrate soln. (4 gm. per 100 ml.)
18.2 ml. A–Z soln. as suggested by HAAS (**6**).

This mixture was diluted to 18 liters with distilled water. The plants were watered sufficiently to provide for their water requirements.

A twelve-day growth period was selected from preliminary experiments as a period during which toxicity to the plant by addition of boron would appear as a necrosis of the leaves.

Soybean seeds of the Black Wilson variety were germinated between sheets of filter paper, and single seedlings transferred to the cultures five days later, when the roots were between four and six centimeters long. Before treatment with boron, the group of experimental plants was re-selected as to uniformity and vigor and the remainder discarded.

Series A and B consisted of eleven treatments each with six plants in each treatment; series E consisted of eleven treatments each containing seven plants; and the series F and G consisted of fifteen treatments with six plants per treatment. The total number of plants in all treatments was 398.

Single additions of boron were made to culture vessels in the form of boric acid in water solution. Treatment in all cases was made by the addition of one ml. of solution in proportion based on the weight of the air dry medium. These were immediately washed into the medium with distilled water to assure uniform distribution of the added boron.

The untreated soil contained 0.27 p.p.m. of water-soluble boron. The boron content of the soybean seeds was 27 p.p.m. on the dry basis. Therefore, none of the plants could be expected to develop a deficiency during the short-term growing period.

The methods used for the determination of boron in both plant and soil were adaptions of the BERGER-TRUOG quinalizarin procedure (**7, 8**).

It was necessary to determine the period of growth during which the treatment should be made. Thus, in the case of the plants in series A (table I), treatment of the young plants with boron was made as the cotyledons opened and the first true leaves appeared. Treatment in other series (B, E, F, G) (tables I and II) was made after the formation of the second true leaves of the plant, ten days after planting.

The effects of boron treatment on the growth of soybean plants grown in sand-soil and in purified sand are shown in table I.

An extreme toxicity appeared in plants receiving 7.5, 10.0, 15.0, and 20.0 p.p.m. boron (in air dry medium) at the appearance of the first true leaves on the plant (series A, table I). All plants in these groups were dead within 24 hours after treatment. The injury first appeared as a withering of the

TABLE I

EFFECT OF BORON TREATMENT ON SOYBEAN SEEDLINGS AT TWO GROWTH STAGES AND IN TWO GROWING CULTURES

Group number	Boron added in medium	Series A — Culture: Purified sand 50%—soil 50% Nutrient solution added				Series B — Culture: Purified sand 50%—soil 50% Traces of Cu, Mn, Zn only				Series E — Culture: Purified sand 100% nutrient solution			
		Average green weight	Average dry weight	Average height	Boron in moisture-free plant	Average green weight	Average dry weight	Average height	Boron in moisture-free plant	Average green weight	Average dry weight	Average height	Boron in moisture-free plant
	p.p.m.	*gm.*	*gm.*	*cm.*	*p.p.m.*	*gm.*	*gm.*	*cm.*	*p.p.m.*	*gm.*	*gm.*	*cm.*	*p.p.m.*
C	0.00	1.17	0.18	11.9	25	1.48	0.31	14.2	24	2.26	0.35	13.2	40
1	0.25	1.20	0.18	13.3	27	1.68	0.34	14.8	41	2.75	0.43	13.7	70
2	0.50	1.41	0.22	14.0	41	1.31	0.27	13.3	74	2.39	0.36	13.5	89
3	0.75	1.38	0.20	15.7	21	1.68	0.35	14.6	60	2.38	0.36	13.1	182
4	1.00	1.19	0.19	12.4	50	1.63	0.34	14.3	113	2.58	0.39	13.2	202
5	2.50	1.14	0.17	12.8	94	1.61	0.29	15.1	210	2.08	0.33	13.5	274
6	5.00	0.98	0.14	11.4	205	1.34	0.23	16.2	663	1.76	0.31	13.5	550
7	7.50	all plants dead 4/18/41				1.30	0.22	16.9	795	1.26	0.26	12.6	777
8	10.00	" " " "				0.77	0.17	16.4	1270	1.23	0.26	12.6	1081
9	15.00	" " " "				0.71	0.17	14.0	1825	0.57	0.20	9.6	2162
10	20.00	" " " "				0.49	0.15	12.4	2582	0.60	0.19	9.6	2669
Plants per group		6				6				7			
Date of planting		4/15/41				4/15/41				4/15/41			
Date of treatment		4/17/41				4/25/41				4/25/41			
Date of harvest		4/29/41				5/ 7/41				5/ 7/41			

TABLE II

EFFECT OF BORON TREATMENT ON SOYBEAN SEEDLINGS GROWN IN PURIFIED SAND AND A MIXTURE OF PURIFIED SAND AND SOIL

Group number	Boron added in medium	Series F, Culture: Purified sand—100% Nutrient solution added				Series G, Culture: Purified sand 50%—soil 50% Traces of Cu, Mn, Zn only			
		Average green weight	Average dry weight	Average height	Boron in moisture-free plant	Average green weight	Average dry weight	Average height	Boron in moisture-free plant
	p.p.m.	*gm.*	*gm.*	*cm.*	*p.p.m.*	*gm.*	*gm.*	*cm.*	*p.p.m.*
C	0.00	1.41	0.24	26.4	56	1.66	0.30	30.2	30
1	0.10	1.45	0.25	27.5	37	1.59	0.29	30.3	30
2	0.20	1.42	0.25	29.2	72	1.62	0.29	33.0	41
3	0.30	1.53	0.27	28.9	84	1.74	0.31	34.7	43
4	0.40	1.63	0.28	29.2	96	1.77	0.31	33.7	40
5	0.50	1.47	0.24	28.3	125	1.64	0.29	33.6	41
6	0.60	1.48	0.25	28.5	109	1.53	0.26	32.0	48
7	0.70	1.60	0.25	29.4	141	1.63	0.28	33.9	51
8	0.80	1.59	0.26	29.3	127	1.83	0.34	34.0	39
9	0.90	1.52	0.26	28.8	138	1.74	0.32	34.9	46
10	1.00	1.43	0.24	29.1	166	1.69	0.31	35.0	50
11	1.25	1.27	0.23	26.7	153	1.66	0.30	35.6	38
12	1.50	1.34	0.24	26.4	199	1.64	0.30	35.0	52
13	2.00	1.19	0.22	26.2	242	1.60	0.29	36.4	79
14	5.00	0.87	0.16	24.7	627	1.48	0.26	34.9	224
Plants per group		6				6			
Date of planting		5/24/41				5/24/41			
Date of treatment		6/ 4/41				6/ 4/41			
Date of harvest		6/16/41				6/16/41			

stem at its base followed by the appearance of a bright red coloration. All other plants receiving 5.0 p.p.m. boron or less survived until harvest.

Treatments subsequent to that of series A were made after the formation of the second true leaves formed on the plant. Therefore, the results indicating the growth response (fig. 1) may not be compared to those of series B, E, F, G, respectively.

Plants grown in cultures containing added concentrations of boron between 7.5 and 20 p.p.m. of boron, but which were treated after the appearance of the second true leaves, were able to withstand the extreme toxic effects of these levels of boron (series B and E, table I) (series F and G, table II).

The first toxic symptoms shown by the plants in series A, other than the immediate reaction to high levels of boron, appeared in group 5 to which

5.0 p.p.m. boron was added. The symptoms were indicated by the appearance of the characteristic necrotic mottling around the edge of the leaf. These appeared on the first true leaves, two days after treatment. At harvest, the injury was found on the first true leaves of four of the six plants on

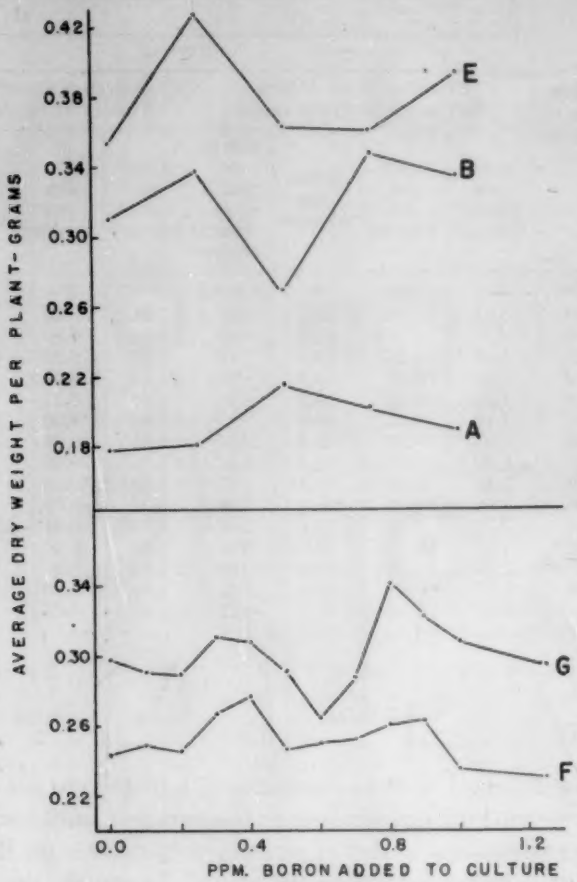

FIG. 1. Growth response to boron treatment.

the 1.00 p.p.m. level of added boron. Yellowing as well as the characteristic mottling of the leaves was very marked at the 2.5 p.p.m. boron level.

The first toxic symptoms on the leaves of plants in series B appeared two days after treatment. Necrotic areas appeared on the first and second true leaves simultaneously at the 10.0, 15.0 and 20.9 p.p.m. level of added boron. At harvest, leaves of all plants receiving more than 2.5 p.p.m. of boron were yellowed and had shown during the growth period definite necrosis of all leaves on the plant. The 2.5 p.p.m. level was the upper limit for production

of normal plants. In later stages of boron toxicity, the necrotic spots were not confined to the leaf margins but appeared over the whole leaf.

Series E (table I) showed some differences in toxic limits as measured by the visible effects on the leaves. Necrotic areas appeared at the edges of the leaves of several plants at levels of 0.5 and 0.75 p.p.m. boron. The 1.0 p.p.m. level was the limit for production of normal plants, while those at the 2.5 p.p.m. level were seriously injured.

A slightly greater dry weight of harvested plant material was found in the case of those grown on the purified sand (series E, table I). The boron content increased more rapidly with boron treatment in the cases where plant growth had been well started before treatment. A maximum of 2669 p.p.m. boron in the moisture free plant material was found as a result of the addition of 20 p.p.m. boron to the purified sand medium.

Two short series using the Virginia Brown variety of soybean and following the plan of series A and B showed similar effects on growth curves and were not included with these data.

Curves drawn from the green weight, dry weight and height of the groups in series A, B, and E, as well as two other short series mentioned above, appeared to show a growth stimulation and maxima in the vicinity of 0.25 p.p.m. of added boron dry media as well as a maximum in the vicinity of 0.75 p.p.m. boron in the cultures. Other investigators have shown (**2, 3, 4**) that such a growth maximum exists in the vicinity of 0.9 p.p.m. added boron for soybean, sunflower, and lettuce plants but have not indicated the existence of such a growth peak at a lower boron concentration. EATON (**5**), in a study of boron toxicity in sunflower seedlings, shows a dry weight curve which indicated maxima at 0.25 and 1.0 p.p.m. boron. He attributed this change to individual variation of the plants.

In order to indicate the results of the previous series in more detail, a second experiment was attempted. Treatments were made to 14 groups of 6 plants each in individual pots with a range of 0 to 5.0 p.p.m. boron. The boron additions were made in the manner previously described, at the appearance of the second true leaves, after which the experiment was continued for a twelve-day growth period. The results of these experiments (series F and G) are shown in table II.

The growth changes were small but the curves of average dry weight with boron content of the cultures (fig. 1) show that a second maximum of growth is present at 0.4 p.p.m. added boron. The duplication of growth maxima in the series B, E, F, and G appear to be conclusive proof that such an effect is present at this stage of growth of the soybean plant. The conditions of treatment for series A differed from that of other series and although stimulation to growth was evident, the growth changes could not be compared with those of series B, E, F, and G.

The relation of boron content of the plant to the growth changes is more evident in series B (table I). Growth stimulation, as measured by dry or green weight increase was followed by an increased boron content of the plant at lower levels of added boron.

Apparently there is a reduction in boron content of plants grown on the sand-soil mixture over that of the sand. This appears to be true more particularly in the second experiment which was started late in May. The plants in these series averaged about twice the height of those grown earlier although the dry weight of plant material was nearly the same. The presence of 200 p.p.m. or more boron in the plant corresponded to the increasing toxicity of the boron to the plant as indicated by foliar symptoms.

Field experiments

A series of field plots were prepared and treated with a row application at the rate of 5, 10, and 15 pounds of borax to the acre. A Latin Square arrangement was used with guard rows between each treatment and that of the untreated plots. Soybeans were used as the experimental plant.

No visible symptoms of toxicity were found in any of the treated plots. Application at the rate of 0, 5, 10, and 15 pounds of borax per acre produced an average yield of 8.7, 8.3, 9.0, and 8.7 pounds of hay, respectively. Although the differences in yield might not be considered great enough to be termed significant, they correspond in effect to growth differences recorded in the greenhouse studies. The average yield of beans was decreased with increased borax application.

An interesting change was found with respect to the boron content of

TABLE III

Variation of boron content of the stem, leaf, and growing tip of field-grown soybean plants during the growing season

Sampling date	Treatment, pounds borax per acre	Boron in dry material		
		Stem	Leaf	Growing tip
		p.p.m.	*p.p.m.*	*p.p.m.*
7/ 5/41	Check	28	64	37
	5	26	68	42
	10	30	78	45
	15	31	100	46
7/19/41	Check	17	59	37
	5	19	52	35
	10	24	64	41
	15	27	56	44
8/ 4/41	Check	17	55	37
	5	16	69	28
	10	14	93	46
	15	18	94	59

portions of the plant cropped at various stages of growth. This is shown in table III. An increase in boron concentration was found in the stems, leaves, and growing tips with increasing boron application, in samples collected July 5, and August 4. In all cases, however, the boron concentration was lower and showed less change upon application in samples collected July 19. No explanation of this difference was evident except that an extremely dry condition existed in this area between the first sampling and several days prior to the last sampling date.

Summary

1. Short-term growth studies on the soybean plant indicate that a twenty-day growth period and twelve-day treatment period are sufficient to demonstrate toxicity of boron both by growth relationships and by foliar symptoms.

2. Visible toxicity to the soybean plant as indicated by foliar symptoms corresponded to the presence of 200 p.p.m. or more boron in the moisture-free plant material.

3. Duplication of growth maxima in four plant series, duplicated in sand and sand-soil mixtures, and treated with varying quantities of boron appears to be conclusive proof that such maxima are present at 0.4 p.p.m. and 0.8 p.p.m. added boron at this stage of growth of the soybean plant. A fifth series (A) showed stimulation to growth but was treated under different conditions.

4. Preliminary field experiments with soybeans indicate a growth change which corresponds in effect to that observed in greenhouse studies.

5. The boron concentration of field-grown soybean plants, at mid season, showed little difference between treatments. The plant at earlier and later growth stages showed increased boron concentration with treatment.

6. This procedure offers a method for testing small quantities of soil for boron toxicity in a relatively short time.

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COMPARISON OF THE CALCIUM AND SULPHUR CONTENT OF PLANT FLUIDS EXPRESSED FROM TISSUES KILLED BY AUTOCLAVING AND BY FREEZING WITH SOLID CARBON DIOXIDE

ALVIN D. AYERS

Sap analysis in combination with analysis of the complete tissue is particularly useful in gaining information concerning the distribution of elements between fluids and solids of a given tissue and the relative availability of elements for conditioning metabolic processes of the plant.

Two methods, killing by heat and killing by freezing with solid carbon dioxide, are readily adapted to the pretreatment of plant tissues in the field for subsequent studies on the expressed sap. Both procedures have been used but few comparisons of results of mineral analyses of sap obtained by the two methods have been published. Results reported in this paper are intended to call to the attention of investigators of plant tissues the possibility of obtaining expressed fluids of different composition depending upon the methods of pretreating the materials. Particular emphasis is placed on the calcium and total sulphur of such saps because these two constituents showed the greatest change as a result of the type of pretreatment for the plants and elements studied. With other plants, other constituents may differ.

BROYER (1) recently reviewed methods for preparing tissues for analysis in physiological studies with plants, but most of the investigators he cited have been primarily interested in the osmotic concentration, the carbohydrate content, or the nitrogen fractions of the expressed saps. Freezing of plant tissues at low temperatures prior to expression of the sap has been studied and used by BROYER and HOAGLAND (2), MASON and PHILLIS (6), GREATHOUSE (4), MEYER (7), and others.

DONEEN (3) found no difference in the freezing point depression, total solids, total sugars, or nitrate-nitrogen in the sap of small wheat plants as a result of freezing or heating. A decrease in the calcium content of sap as a result of heating has been noted by ILJIN (5) for several different plants. While studying the "partition indices" of potassium, magnesium, and calcium in cotton leaves, PHILLIS and MASON (9) noted a difference in the concentrations of potassium and calcium between sap expressed from frozen and from boiled leaves and attributed this to the occurrence of base exchange during boiling.

Experimental technique

Tissues from cotton and tomato plants grown in nutrient solutions containing added chloride and sulphate salts were chopped into pieces approximately one centimeter or less in length, mixed thoroughly, and divided into equal parts. Each portion was sealed in a pint glass fruit jar. One series was placed in contact with "dry ice" for 16 to 20 hours in a small

refrigeration box and then stored in a cold storage locker at 6° to 8° F. until time of analysis. The other series was autoclaved immediately at 15 pounds pressure for 15 minutes. After thawing, the frozen samples were subjected to a pressure of 2,000 pounds per square inch in a Carver Press. Identical pressing technique was used on all samples. The expressed juices were centrifuged at 1500 r.p.m. for 5 minutes.

TABLE I

CALCIUM AND TOTAL SULPHUR ANALYSES OF PRETREATED PLANT TISSUES

SAMPLE DESCRIPTION	MILLIEQUIVALENTS PER LITER OF SAP			
	CALCIUM		TOTAL SULPHUR AS SULPHATE	
	FROZEN	AUTOCLAVED	FROZEN	AUTOCLAVED
	m.e./l.	*m.e./l.*	*m.e./l.*	*m.e./l.*
Cotton leaves				
1	263	208	.....	.....
	.....	200	.....	.....
2	251	230	.....	.....
3	241	192	.....	.....
4	381	211	183	135
	388	212	194	132
5	397	223	164	106
	.....	214	.....	108
	.....	211	.....	103
6	421	227	167	103
7	427	249	154	94
	457	249	155	97
8	352	273	236	207
	350	248	233	197
Tomato leaves				
A–1	64	57	148	129
A–2	56	56	155	132
A–3	45	43	179	129
A–4	25	27	183	165
B–2	49	51	116	103
B–3	49	46	176	119
B–4	30	35	182	163

Results

Table I presents the calcium and total sulphur analyses of expressed saps from cotton and tomato tissues which were pretreated by freezing and by autoclaving. Duplicates shown represent portions of the original material carried through the entire procedure including the pretreatment. Standard analytical procedures were used.

Autoclaving before pressing cotton leaves yielded a sap which had less calcium and less total sulphur than the sap from leaves killed by freezing. The total sulphur content of sap expressed from tomato leaves and young stalks was likewise lower from the autoclaved than from similar frozen tissues. The calcium content, in this latter case, was not affected by the method of pretreatment and was lower than that from the cotton.

Other constituents (Mg, K, Na, and Cl) were also determined but are not shown since differences due to treatment were generally small and not conclusive. Total chlorine content of the saps obtained by the two methods agreed closely. This indicates that there were no concentration changes which could be accounted for by a differential loss of water as a result of the type of pretreatment.

Table II shows the partial composition of sap expressed from cotton leaves subjected to two additional treatments. One set of samples was

TABLE II

EFFECT OF TREATMENT OF COMPOSITION OF SAP OF COTTON LEAVES

TREATMENT	MILLIEQUIVALENTS PER LITER OF SAP		
	CALCIUM	TOTAL SULPHUR	TOTAL CHLORINE
Frozen	352	236	20
	350	233	19
Autoclaved	261	195	19
	244	185	20
Frozen and autoclaved	273	207	19
	248	197	19
Frozen, expressed and autoclaved	229	168	20
	217	168	20

frozen as previously described; a second set was autoclaved; a third set was frozen and then autoclaved; and the fourth set was frozen and thawed, the sap expressed and centrifuged, and then autoclaved. All saps were centrifuged and analysis started immediately after expression. When the frozen sap was autoclaved, a coagulated mass settled to the bottom of the container. This coagulum was removed during the centrifuging and when analyzed was found to be high in both calcium and total sulphur.

Discussion

Killing by any method whatsoever irreversibly alters the plant cells. The semipermeability of the protoplasmic membranes is destroyed, dissipating the concentration gradients previously set up within the cell and between adjacent cells. Soluble constituents of the protoplasm may then mix with constituents of the vacuole and those from one cell with those of other cells which may be quite different histologically and in their accumulated salts. With the redistribution of ions within and between cells, some insoluble salts may go into solutions, some salts may be precipitated, and a new exchange equilibrium be established. Fluids expressed from such tissues may then contain quantities of certain ions which are different from the total soluble amounts present in the uninjured cells.

Many organic colloids exert a protective action in preventing the precipitation of compounds whose solubility has exceeded that found for simple solutions. Some similar type of mechanism probably operates within the plant. Evidence shows that heating coagulates most all plant proteins but that freezing does not appear to alter irreversibly their dispersibility in the tissue fluids. Any coagulation which occurs destroys the protective action of these organic colloids and in this way could cause precipitation within the tissues of compounds which formerly were protected.

Coagulation of proteins may reduce the mineral content of the sap by the amount present in the proteins. The relative importance of this effect will depend upon the percentage of the mineral associated with the protein. Besides sulphur and phosphorus, calcium, magnesium, and sodium may also be combined with the protein.

ILJIN (5) suggested that calcium citrate may be precipitated by heating and thus lower the calcium content of saps pressed from heat-killed plants. Data for cotton given in tables I and II show reduction in total sulphur as well as calcium. This suggests that calcium sulphate may have been precipitated by autoclaving. The stoichiometrical reduction of calcium was higher, however, than that for sulphur indicating that calcium was removed by some other means such as precipitation as the citrate or some other insoluble form.

PHILLIS and MASON (9) also found that the calcium content of saps expressed from heated cotton leaves was lower than that expressed from the frozen leaves. This reduction on heating was attributed to the occurrence of base exchange reactions during the heating. Such base exchange reactions could account for small changes in concentration but it is doubtful if they could account for a reduction of over 100 milliequivalents of calcium per liter of sap as shown in tables I and II. Furthermore, no such increases or decreases in any of the other bases were found. If this were a true base exchange reaction, magnesium, sodium, and potassium equilibria would also be expected to undergo proportionate stoichiometric changes. Likewise, it would be difficult to account for the decrease in total sulphur by anion exchange when there was no increase or decrease in the total chlorine content of the saps upon heating.

Pretreatment by freezing or heating allows a large percentage of the plant juices to be pressed from the plant tissues and has been used extensively. Such sap is not comparable to the plant fluids as they exist in the vacuole and cytoplasm of unkilled cells (9) but is a composite of the fluids occurring within these tissues. And, as has been shown, the composition of this composite liquid may be altered by the pretreatment of the tissues prior to pressing. Because of possible precipitation of certain constituents in the sap during heating, killing by severe freezing prior to pressing is the preferable procedure.

Summary

1. The concentration of several mineral constituents in saps expressed from plant materials pretreated by freezing with solid carbon dioxide and by autoclaving were compared.

2. Calcium and total sulphur contents of the saps expressed from autoclaved cotton leaves were lower than those of saps expressed from similar samples killed by freezing.

3. Total sulphur content of saps expressed from autoclaved tomato leaves and stalks was lower than that expressed from similar samples killed by freezing.

4. Precipitation of calcium rather than a base exchange reaction during the autoclaving is indicated.

5. Killing of plant tissues by severe freezing prior to the removal of the fluids by pressing is preferable to killing by heat.

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INTERLABORATORY COMPARISON OF ABSORPTION SPECTRA BY THE PHOTOELECTRIC SPECTROPHOTOMETRIC METHOD.—DETERMINATIONS ON CHLOROPHYLL AND WEIGERT'S SOLUTIONS[1]

F. P. ZSCHEILE, C. L. COMAR AND G. MACKINNEY

Since numerous differences in both absolute and relative values of absorption coefficients as well as in wavelengths of maxima for chlorophyll solutions have been summarized recently in the literature (**1, 3**), it seemed desirable to make certain comparative tests on the same materials measured by similar methods in different laboratories. Experiments were designed to determine the extent to which instrumental differences might account for those discrepancies observed by different workers employing the photoelectric spectrophotometric method. To this end, Weigert's solution and chlorophyll were selected, the former because of its stability and previous evaluations of its absorption, the latter because of our primary concern with this pigment.

Experimentation

Each of the two laboratories cooperating in this study employs a double monochromator optical system in the photoelectric spectrophotometer with which the measurements were made. The performance of the Purdue instrument has been evaluated relative to chlorophyll spectra (**3**). The instrument employed by MACKINNEY has been described by SMITH (**2**).

WEIGERT'S SOLUTION.—ZSCHEILE and COMAR prepared Weigert's solution consisting of equal volumes of 0.100 M solutions of $CuSO_4$ and K_2CrO_4 in 1.99 N NH_4OH solution. They sent aliquots of this Weigert's solution, sealed in Pyrex tubes, packed in dry ice, to MACKINNEY for study. The same solvent (1.99 N NH_4OH solution) was sent for the solvent-filled cell. Results are tabulated in table I together with results previously obtained. Cell lengths of 2 cm. were employed throughout.

CHLOROPHYLLS.—Measurements were made on chlorophyll *a* prepared by the method of ZSCHEILE and COMAR (**3**) and on both components prepared by MACKINNEY's procedure (**1**).

a. On November 19–20, 1940, chlorophyll *a* was isolated by ZSCHEILE and COMAR in ether solution [eluted directly from the adsorption column (**3**)]. Appropriate dilutions for the spectroscopic measurements were made from the original solution and aliquots were shipped in tightly corked bottles, packed in dry ice, by air express to MACKINNEY who studied them on Novem-

[1] Journal Paper no. 4, Purdue University Agricultural Experiment Station. Herman Frasch Foundation for Research in Agricultural Chemistry, Paper no. 215.

TABLE I

MOLECULAR ABSORPTION COEFFICIENTS* FOR WEIGERT'S SOLUTION

WAVELENGTH IN Å		SOLUTION PREPARED AT PURDUE			ABSORPTION COEFFICIENTS	
		CONCENTRATION	ABSORPTION COEFFICIENTS		PREVIOUS CALIFORNIA VALUES	
CALIFORNIA	PURDUE		CALIFORNIA (22° C.)	PURDUE (25° C.)	(1940)	(1936)‡
Å	*Å*					
5300	.....	c/4†	27.1	.....	26.8	26.95
5200			21.9	.....	22.1	21.95
5100	5106		17.6	18.9	17.7	17.75
5000	5004		14.6	16.0	14.9	14.95
4900	4903		14.8	16.4	15.4	15.50
4800	4802		22.0	23.3	22.8	23.45
7000	7026	c/10	42.9	42.5	42.5	41.05
6800	6824		48.3	47.8	47.9	46.70
6700	6723		50.9	50.4	50.5	49.45
6500	6521		55.8	55.5	55.0	54.25
6300	6319		58.3	58.4	57.7	57.05
6200	6218		58.6	58.9	57.9	57.40
6100	6116		58.4	58.6	57.6	57.25
6000	6015		57.1	58.3	56.3	55.80
5800	5813		52.1	53.1	50.9	50.60
5600	5611		43.7	45.1	43.8	42.60
5400	5410		33.2	34.6	32.6	32.50
.....	5308		.....	29.6	.....	.....
5200	5207		22.6	24.4	22.1	21.95
4800	4802		22.6	24.5	22.8	23.45
4700	4702		42.7	44.0	42.6	43.5
4600	.....		84.0	.....	85.8	86.0
4600	4601	c/40	84.0	88.5	85.8	86.0
4500	4501		156.	161.	161.2	159.3
4400	4400		259.	264.	265.7	264.5

* Calculated equivalent to E (2).
† c = 0.05 molar.
‡ Reference (2).

ber 22. A sample of ether for the solvent-filled cell was included also. This chlorophyll had not been dried, as was found desirable in previous work (**3**). Previous data have shown that ether solutions of chlorophyll which are not exposed to heat or light are stable for at least three days. In the meantime, aliquots of the original solution were dried as described by ZSCHEILE and COMAR (**3**) for determination of concentration. After receiving the spectrophotometric data from MACKINNEY, the concentration value was sent to him and the absorption coefficients calculated from the data of the two laboratories. Comparative results are presented in table II. The slits of the Purdue instrument were 0.06 mm. wide. MACKINNEY employed slits 0.07 mm. wide. Information on the spectral region isolated may be obtained from earlier papers (**2, 3**). All results presented were obtained with an incan-

TABLE II

SPECIFIC ABSORPTION COEFFICIENTS* FOR CHLOROPHYLL *a* IN ETHER SOLUTION (PREPARED AT PURDUE)

WAVELENGTH	CONCENTRATION†	ABSORPTION COEFFICIENTS‡	
		CALIFORNIA (22° C.) (Nov. 22, 1940)	PURDUE (25° C.) (Nov. 21, 1940)
A			
6620	c/196	99.7	98.0
6610		101.8	100.2
6600		100.8	100.8
4320		122.	122.
4300		130.	132.
4280		130.	131.
4260		122.	125.
6350	c/28	9.75	10.1
6330		9.75	9.87
6180		15.5	15.2
6160		15.7	15.6
6140		15.6	15.8
5900		6.16	6.19
5800		8.55	8.50
5050	c/4	1.92	1.92
4740		0.982	0.965
4720		0.928	0.932
4700		0.923	0.932
4680		0.942	0.948

* Calculated equivalent to *a* (3).
† Concentration c = 498 mg./l.
‡ Cell length = 2 cm.

descent lamp source. No difference was found by MACKINNEY when slits were made 0.05 mm. wide at wavelengths 6600 and 4300 Å.

b. In January, 1941, MACKINNEY sent dried samples of chlorophylls *a* and *b* (prepared in November, 1940) to ZSCHEILE and COMAR, sealed in evacuated glass tubes, at ordinary temperatures. They were prepared by the method described by MACKINNEY (1). The evacuated tubes were broken in transit but the samples were not contaminated with any visible particles. COMAR and ZSCHEILE then studied these samples spectrophotometrically without knowledge of MACKINNEY's results. Comparison with measurements made by MACKINNEY on these same samples is made in table III. Both ether and 80 per cent. acetone were employed as solvents for these solid samples. Instrumental conditions were the same as in part a.

Discussion

It is evident from the nature of the absorption curves for chlorophyll *a* (3) and for Weigert's solution (2) that a comparison of data on the former from different laboratories is a more critical test of instrumental factors

TABLE III

TABLE III

SPECIFIC ABSORPTION COEFFICIENTS* FOR DRIED CHLOROPHYLLS
(PREPARED IN BERKELEY)

ABSORPTION COEFFICIENTS IN 80 PER CENT. AQUEOUS ACETONE			ABSORPTION COEFFICIENTS IN ETHER		
WAVELENGTH Å	CALIFORNIA‡ (20–22° C.)	PURDUE§ (25° C.)	WAVELENGTH† Å	CALIFORNIA‡ (20–22° C.)	PURDUE‡ (25° C.)
Chlorophyll *a*					
	(JAN. 2, 1941)	(JAN. 27, 1941)		(JAN. 2, 1941)	(FEB. 5, 1941)
6700	56.75	64.5	6600	93.4	98.4
6630	82.0	81.9	6400 (6390)	11.2	12.09
6600	76.0	74.9	6350	9.57	10.00
6500	28.5	31.3	6300 (6310)	10.16	9.94
6350	11.62	12.48	6200 (6187)	13.74	14.29
6200	16.37	17.05	6100 (6106)	13.96	14.80
5800	9.02	9.70	5800 (5803)	7.66	7.85
5400	3.44	3.84	5300	3.52	4.05
5350	3.49	3.83	4800	1.12	1.26
5000	1.75	2.10	4720	1.10	1.18
4800	1.30	1.56	4600	1.80	1.87
4600	3.19	3.07	4500 (4520)	6.41	3.55
4400	58.62	61.0	4300	116.5	128.2
4300	90.78	91.8	4200	80.2	79.8
Chlorophyll *b*					
	(JAN. 3, 1941)	(JAN. 28, 1941)		(JAN. 3, 1941)	(FEB. 8, 1941)
6600	14.69	14.96	6450 (6455)	50.82	50.0
6450	45.60	47.4	6420 (6425)	53.58	55.1
6350	20.32	24.8	6200 (6212)	7.02	8.04
6200	9.06	9.80	6100 (6110)	7.62	8.00
6150	9.00	9.68	5900 (5907)	11.59	10.40
5800	7.80	8.61	5200	2.97	3.02
5400	5.33	5.92	5100	2.92	2.64
5200	3.08	3.74	5050 (5060)	2.66	2.61
5000	4.02	4.54	4900	4.34	2.78
4800	28.00	30.2	4550	142.2	154.0
4700	82.41	87.3	4500	128.5	149.0
4600	130.3	136.7	4400	62.23	67.5
4500	90.6	96.0	4300	54.0	58.4

* Calculated equivalent to α (3).
† Where identical wavelengths were not employed, the nearest, in parentheses, apply to the Purdue column.
‡ Cell length = 2 cm.
§ Cell length = 1 cm.

than a similar comparison of data on Weigert's solution. A given amount of scattered radiation would alter the values of the absorption coefficients at the maxima and minima much more when the bands are high with steep slopes than when they are extremely broad with comparatively gradual slopes.

When the data on Weigert's solution are plotted, only minor differences are observed. It is probable that small spectroscopic differences may arise from a variation in NH_3 concentration which is difficult to control during the manipulations required. The agreement is considered satisfactory and the differences observed are considered to be due chiefly to unavoidable minor variation in the solutions.

The excellent agreement of the data on chlorophyll *a*, table II, both with respect to absorption values and to wavelengths of maxima and minima, shows that instrumental differences need not be considered further in the comparison of chlorophyll spectra measured by these two instruments. Such differences as are found must be inherent in the samples studied.

It is noted that in aqueous acetone, table III, the maximum values at 6630 and 4300 Å for chlorophyll *a* are virtually identical on the two instruments and that for the *b* preparation, the ratios of the values at 6450 and 4600 Å are the same. In ether solution, agreement at the maxima is not as good as in aqueous acetone but agreement is considerably better in the former solvent at the minimum of absorption.

This comparative study has served to indicate the agreement of results obtainable by photoelectric spectrophotometric procedure on two instruments and that discrepancies in the chlorophyll absorption are relatively minor in comparison with those reported during the last ten years.

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INFLUENCE OF SULFANILAMIDE AND DERIVATIVES ON GROWTH OF *LUPINUS ALBUS*

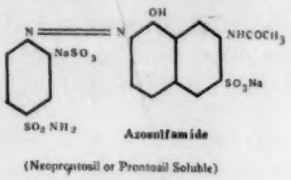
DAVID I. MACHT

Introduction

The marvelous therapeutic results obtained during the past few years in various infections with sulfanilamide and its derivatives, originating in the epoch-making discoveries of DOMAGK, have naturally stimulated pharmacological and bacteriological work on these compounds. The pharmacological and toxicological experiments with the sulfamides, however, have been almost exclusively of a *zoo*pharmacological nature, that is, confined to experiments on animals and animal tissues. As these compounds are primarily chemotherapeutic agents affecting in some still inexplicable manner the growth of bacteria, and as bacteria are actually more closely related to lower plants than to lower animals, the writer, who has been making *phyto*pharmacological studies for many years, determined to investigate the effect of various sulfa-compounds on living plants. The results already obtained are of both theoretical and practical interest. The present paper is a report on the influence of sulfa-drugs on root growth of *Lupinus albus* seedlings.

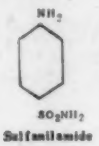
Compounds studied

The compounds studied were (1) sulfanilamide, (2) neoprontosil, (3) sulfathiazole, (4) sulfapyridine, (5) sulfadiazine, (6) sulfaguanidine and (7) succinyl sulfathiazole,[1] the structure of which is shown in the subjoined formulae. There was a difference in the solubility of the respective com-

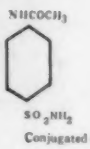


Azosulfamide

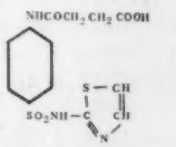
(Neoprontosil or Prontosil Soluble)



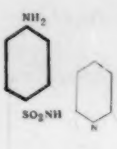
Sulfanilamide



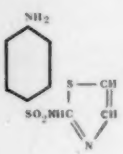
Conjugated or Acetylated Sulfanilamide



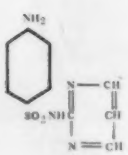
Succinyl Sulfathiazole



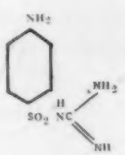
Sulfapyridine



Sulfathiazole



Sulfadiazine



Sulfaguanidine

[1] Made by Sharp & Dohme and secured through the courtesy of Dr. EDGAR J. POTH.

pounds in water and plant-physiological saline but each was sufficiently soluble to admit of the study of seedling growth in concentrations varying from 1 : 10,000 to 1 : 500,000.

Method of study

Seeds of *Lupinus albus*, large variety, were soaked in tap water overnight and planted in finely divided sphagnum moss containing sufficient moisture. After germination, the growth of selected seedlings with roots 35 to 50 mm. long was studied in a solution containing equal parts of distilled water and SHIVE (7) physiological saline. In these studies, the various sulfa-drugs were dissolved in the same medium, *i.e.*, half SHIVE solution and half distilled water. At least ten seedlings, placed in rows of hard-glass test tubes, were employed for the control solution and also for each solution to be studied. The sharply defined straight roots of the seedlings were measured at the beginning and end of every experiment and the ratio of growth of the seedling roots in the various sulfa-solutions was expressed as a percentage of their growth in the control solution according to the formula, index of growth $= \frac{X}{N}$, in which N represents average growth of controls and X the average growth in solution containing the drug to be tested. All of the seedlings were allowed to grow for 24 hours in the dark at 20° C. Further details regarding technique, which are not essential here, have been published elsewhere (4).

Results

Ten experiments were made with each drug and the average figures obtained with the respective compounds are cited.

EFFECT OF CONCENTRATIONS OF 1 : 10,000.—Four of the compounds, *i.e.*, sulfanilamide, sulfapyridine, sulfathiazole and sulfadiazine, in solutions of 1 : 10,000, slightly inhibited root growth of *Lupinus albus* seedlings. The other three compounds, namely, sulfaguanidine, succinyl sulfathiazole and

TABLE I

EFFECT OF 1 : 10,000 AQUEOUS SOLUTIONS OF RESPECTIVE SULFA-COMPOUNDS ON LUPINUS SEEDLINGS

DRUG ADMINISTERED	PHYTOTOXIC INDEX
	%
Sulfanilamide	83
Sulfapyridine	88
Sulfathiazole	86
Sulfadiazine	87
Sulfaguanidine	114
Succinyl sulfathiazole	113
Neoprontosil	137

neoprontosil, in the same concentration, stimulated root growth as shown in table I.

GROWTH IN HIGHER DILUTIONS OF THE DRUGS.—The results obtained with concentrations of 1:10,000 logically suggested that the chemicals exerted that diphasic action on plant growth which is so often observed in phytopharmacological experiments. Effects of such high dilutions are shown in table II. It will be seen that even the first four compounds, which in solutions of 1:10,000 were slightly toxic for root growth of *Lupinus albus*, exerted a stimulating action when employed in concentrations of 1:250,000.

TABLE II

EFFECT OF HIGHER DILUTIONS OF AQUEOUS SOLUTIONS OF THE VARIOUS SULFA-COMPOUNDS ON GROWTH OF LUPINUS SEEDLINGS

DRUG	CONCENTRATION	PHYTOTOXIC INDEX
		%
Sulfanilamide	1: 10,000	83
"	1: 100,000	86
"	1: 250,000	112
Sulfathiazole	1: 10,000	86
"	1: 100,000	90
"	1: 250,000	118
Sulfapyridine	1: 10,000	88
"	1: 100,000	94
"	1: 250,000	130
Sulfadiazine	1: 10,000	87
"	1: 100,000	113
Sulfaguanidine	1: 10,000	114
Succinyl sulfathiazole	1: 10,000	113
Neoprontosil	1: 10,000	137

Effect of blood sera containing sulfa-compounds

Since ordinarily in medical practice all the sulfa-drugs are given by mouth and their concentration or so-called drug level in the blood serum is followed in clinical laboratories, the author investigated the effect of normal blood sera, as well as of those obtained from rabbits and human patients treated with the various compounds in hospitals. In studies on rabbits doses of 0.5 to 1.0 gram were administered to the animals by stomach tube. Samples of blood were obtained by directly puncturing the heart six and twenty-four hours after administration of the drugs. The pharmacological properties of one per cent. of the sera separated from such blood samples were studied in plant-physiological solution. Prior researches of the writer and his associates on thousands of normal animals have shown that the phytotoxic index of the blood of mammals (including rabbits) varies from 70 to 75 per cent. (**3**). Table III shows the effect of one per cent. solutions

of rabbit sera after administration of the various compounds on seedlings studied in the manner described. It will be seen that none of the com-

TABLE III

EFFECT ON LUPINUS SEEDLINGS OF 1 PER CENT. SOLUTIONS OF SERA OF RABBITS RECEIVING 0.5 GM. OF RESPECTIVE SULFA-COMPOUNDS

DRUG ADMINISTERED	PHYTOTOXIC INDEX
	%
Sulfanilamide	79
Sulfapyridine	86
Sulfathiazole	81
Sulfadiazine	83
Sulfaguanidine	79
Succinyl sulfathiazole	81
Neoprontosil	96

pounds exerted any greater toxicity than normal rabbit sera; in fact, some of them were definitely less toxic. Table IV shows the results obtained with

TABLE IV

EFFECT ON LUPINUS SEEDLINGS OF 1 PER CENT. SOLUTIONS OF SERA OF PATIENTS TO WHOM RESPECTIVE SULFA-COMPOUNDS HAD BEEN GIVEN

DRUG ADMINISTERED	PHYTOTOXIC INDEX
	%
Sulfanilamide	76
Sulfapyridine	94
Sulfathiazole	77
Sulfadiazine	85
Sulfaguanidine	83
Succinyl sulfathiazole	78

one per cent. solutions of blood sera obtained from patients treated with the various sulfa-compounds. Here again, it will be seen that the phytotoxic index for *Lupinus albus* seedlings was definitely higher than that of normal human serum, which also varies from 70 to 75 per cent.

Discussion

The hydroponic experiments described in the first part of this paper are of interest in connection with the mechanism of action of sulfa-compounds in human therapeutics. It may be stated that generally the antiseptic potency of drugs with regard to both bactericidal and bacteriostatic effect runs parallel to their toxicity for higher forms of plant life than bacteria; in fact, various fungi, *e.g.*, yeasts, have been employed for roughly testing the antiseptic properties of different drugs. It is well known that the *modus operandi* of the sulfamides is not quite clear and that they are not efficient

therapeutic agents by virtue of a direct bactericidal action. The plant-physiological experiments described in the foregoing paper afford additional evidence of the complexity of the whole subject, which has been so well presented by LONG (**2**).

The serological experiments described in the foregoing communication are of considerable clinical interest. MACHT and PELS (**5**) some years ago described the phytotoxic properties of blood sera obtained from all kinds of dermatoses or skin diseases. The sera from most skin diseases do not differ in toxicity from normal human sera. A striking exception to this rule is the grave and baffling skin disease known as pemphigus. The blood sera from pemphigus patients exhibits a marked toxicity for growth of *Lupinus albus* seedlings under standardized conditions, and the phytopharmacological test has been of great help in establishing a correct diagnosis of pemphigus in its early states (**6**). During the past ten years bloods from nearly three thousand patients suspected of having pemphigus have been tested phytopharmacologically, and although the sera were thus examined without previous knowledge of the patients' histories, the diagnosis made on this basis has almost invariably been confirmed by subsequent histories of cases studied. Now it is well known that all the sulfamide drugs frequently effect dermatological reactions. In the last few years, a number of investigators have reported occurrence of bullous eruptions simulating pemphigus after administration of various sulfa drugs (**7, 8**). The question arises: Are such pemphigoid lesions an indication of true pemphigus? The current study answers this query categorically and considers a convenient method for differentiating between true pemphigus and pemphigoid dermatoses following administration of sulfanilamide and its derivatives.

Summary

1. The effect of sulfanilamide and six of its derivatives was studied on root growth of *Lupinus albus*.

2. Hydroponic solutions containing the drugs exerted a diphasic action, concentrations of 1 : 10,000 inhibiting and high dilutions of 1 : 250,000 stimulating growth of roots of *Lupinus albus*.

3. Blood sera of rabbits and human beings after administration of the sulfa-drugs are no more poisonous than normal blood sera and are usually less toxic.

4. Phytopharmacological examination of such sera affords a convenient method of differentiating true pemphigus from pemphigoid manifestations of the skin produced by the sulfa-drugs.

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A COMPARISON OF THE EFFECTS OF GREEN LIGHT AND OF RED LIGHT ON THE SIMPLE-LEAF DEVELOPMENT OF INTACT AND DECAPITATED BEAN PLANTS

LEWIS H. FLINT AND CHARLES F. MORELAND

(WITH ONE FIGURE)

Increasing effort is being directed to the study and interpretation of both quantitative and qualitative effects of light on plants and a number of investigators have emphasized the inadequacy of present information. Any satisfactory description of the ways in which light may affect the form and growth of plants may be anticipated only as a result of many small contributions by various investigators using varied angles of approach.

At the Richmond meeting in 1938 a report was made on experiments in which white potatoes, beans, and strawberry plants were grown for some weeks in continuous illumination under green fluorescent mercury tube grids. The point of seeming interest at that time was that while the plants made fair growth, no starch was found at any time in the leaf mesophyll, though it was abundant in the guard cells. That starch was abundant under such conditions afforded supporting evidence that the guard cells were without plasmodesmata. The results suggested the possible usefulness of this type of illumination when stomata counts were desired (figure 1), since upon clearing with alcohol and staining with IKI solution the stomata became very prominent. The relations of quality of light to stomatal opening and to translocation were obscure and no interpretation of the plant response under this illumination was advanced.

During 1941 two sets of tests with this illumination were carried out. In the first of these the growth of the simple leaves of beans (*Phaseolus vulgaris* L.) under continuous green light was compared with the growth under continuous red light from neon tube grids. All plants were started in April in cans of soil in a greenhouse and were transferred to the experimental conditions at a time when the simple leaves were about half expanded and small compound leaves were just beginning to expand. Sixteen or more plants were placed under green and red tube lights in a chamber in which a temperature of 20° C. was maintained. Other groups of plants were placed under similar lights in a laboratory in which room temperatures of about 25° C. prevailed. Under each set of conditions half of the plants of each lot were decapitated just above the simple leaves. In addition to the plants under green and red tube lights a further set was placed under green G. E. fluorescent lamps at room temperature. Following one week of continuous illumination the simple leaves were measured. The results obtained have been brought together in table I.

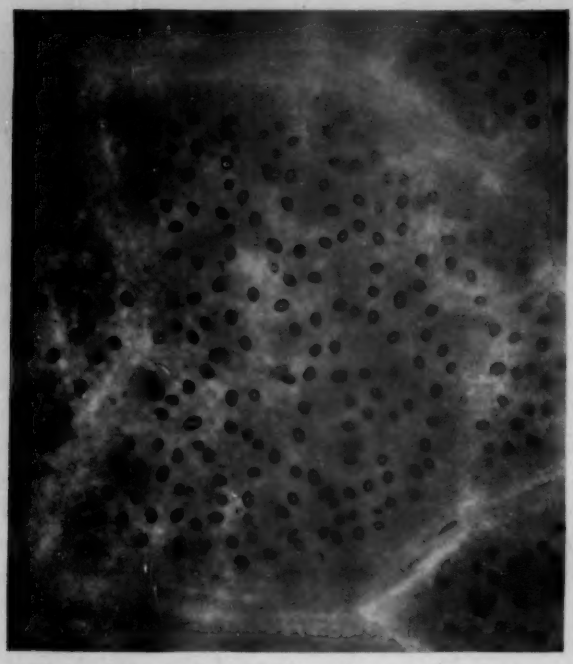

FIG. 1. Photomicrograph showing starch-filled guard cells of stomata in leaves developed under green fluorescent tube lights.

TABLE I

RESULTS OBTAINED IN A STUDY OF THE DEVELOPMENT OF THE SIMPLE LEAVES OF BEAN PLANTS GIANT STRINGLESS VARIETY UNDER DIFFERENT CONDITIONS. AVERAGES OF 16 MEASUREMENTS IN EACH CASE

TREATMENT		MEASUREMENTS		
CONTINUOUS ILLUMINATION FOR ONE WEEK		LENGTH OF SIMPLE LEAVES		WIDTH OF SIMPLE LEAVES
		PETIOLES	BLADES	
		cm.	*cm.*	*cm.*
RED LIGHT				
Neon tube 20° C.	intact	2.50	5.33	5.83
	decapitated	3.96	6.96	7.16
Neon, about 25° C.	intact	2.12	5.08	5.43
	decapitated	3.50	7.12	6.91
GREEN LIGHT				
Fluorescent tube, 20° C.	intact	2.66	5.16	5.58
	decapitated	2.25	4.75	5.50
Fluorescent tube, about 25° C.	intact	2.16	4.33	4.50
	decapitated	2.00	3.75	4.41
G. E. fluorescent lamp, about 25° C.	intact	2.66	5.91	6.00
	decapitated	2.58	5.50	5.91

The results given in table I indicated that under the red light the simple leaves of the decapitated plants were larger than those of the intact plants while under the green light the relationship was reversed. What else the results indicated was not clear. If interpretation were ventured with intensity of light and not quality of light as the major influence, differences in degree but not in direction were expected. If interpretation were ventured with quality of light and not intensity of light as the major influence, more marked differences between the intact plants were expected.

In a second set of tests, carried out in September, the procedures were about the same. A different variety of bean was used and more plants were included in each treatment. The results have been brought together in table II.

TABLE II

RESULTS OBTAINED IN A STUDY OF THE DEVELOPMENT OF THE SIMPLE LEAVES OF BEAN PLANTS KENTUCKY WONDER WAX VARIETY UNDER DIFFERENT CONDITIONS. AVERAGES OF 20 TO 60 MEASUREMENTS IN EACH CASE

TREATMENT		MEASUREMENTS		
CONTINUOUS ILLUMINATION FOR ONE WEEK		LENGTH OF SIMPLE LEAVES		WIDTH OF SIMPLE LEAVES
		PETIOLES	BLADES	
RED LIGHT				
Neon tube, 15° C.	intact	2.905	4.61	5.29
	decapitated	3.625	5.31	6.10
G. E. red fluorescent lamp, about 25° C.	intact	3.08	4.03	4.75
	decapitated	3.46	4.90	5.67
GREEN LIGHT				
Green fluorescent mercury tube 15° C.	intact	2.94	4.40	5.54
	decapitated	2.91	4.35	4.70
G. E. green fluorescent, about 25° C.	intact	3.42	5.08	5.47
	decapitated	3.12	5.00	5.08

In connection with the interpretation of the results indicated in tables I and II it was thought that starch tests of the leaves under the various conditions might prove serviceable. Leaves of plants under the green light had abundant starch in the guard cells, but none in the mesophyll. Leaves of plants under the red light had starch in both guard cells and mesophyll, but starch was abundant only in the mesophyll of the simple leaves of the decapitated plants.

Inasmuch as the results given in tables I and II seemed to be in satisfactory agreement, a consideration of the collective data seemed warranted. The development of the simple leaves of the intact plants was slightly greater under green light than under red light. Under green light the development of these leaves in the decapitated plants was slightly less than in the intact

plants. On the other hand, the development of the simple leaves of the decapitated plants was appreciably greater under red light than under green light. Under red light the development of these leaves in the decapitated plants was appreciably greater than in the intact plants. It would appear to follow that the "normal" or "to be anticipated" responses of the simple leaves to the two sorts of illumination were abruptly reversed incident to decapitation. That red light had a positive effect in bringing about development was evidenced by the fact that simple leaves of decapitated supplementary control plants placed in darkness failed to make any corresponding development.

There seems to be no satisfactory explanation of the observed reversal of response and stimulation at the present time. The sources of illumination used in the experiments were standard types with established spectral distribution characteristics and in each comparison set the plants were placed at the same distance from the light source. Increasingly it has come to be realized, however, that intensities of differing radiations, whether measured with a photronic cell or with a thermocouple, are of uncertain service in the interpretation of plant responses. A little energy at one wave-length may be so effective as to offset or reverse a plant response otherwise associated with the peak output of energy. If the light factor be disregarded, one may say that the plant responses were associated with the shock of injury; with the production of wound hormones; with the cessation of an inhibiting action of apically-produced hormones; with the modification of the functioning of the translocation system following decapitation; or with some other factor or complex. Yet the fact that red light was so effective in promoting development under such modified conditions suggests the involvement of chlorophyll —the only pigment known to be present which absorbed red light.

There has been some evidence that growth substances analogous to those originating in apical meristem may induce a temporary stimulation of photosynthesis, followed by retardation. Mitchell and Hamner (**4**), working with decapitated bean plants, reported that within several days such plants treated with indole-3-acetic acid accumulated an appreciably greater amount of dry matter. Cholodny and Gorbovsky (**2**), working with pairs of leaves from various plants, concluded that this growth substance caused a temporary intensification of photosynthesis—followed by a decrease. Such a type of action might well be involved in the rise and decline characterizing leaf elaboration, and the revival of this sequence following decapitation.

On the other hand, there has been some evidence that renewal of chlorophyll activity following decapitation might center around food supply. Bennett (**1**), in studying virus movement, found that defoliation (with attendant injury) modified the distribution and the rate of distribution of viruses—assumed to travel with the food reserves. Hildebrand (**3**) con-

cluded from experiments designed to enhance the transmission of viruses that pruning temporarily changed the course of the translocation stream as evidenced by the appearance of virus disease symptoms in new leaves.

Summary

In young bean plants grown under green mercury-incited powder tubes and fluorescent lamps there was satisfactory growth accompanied by an accumulation of starch in the guard cells without a corresponding development in the mesophyll. Under these conditions the development of the simple leaves was slightly greater than in red light obtained from indicated sources. Following decapitation there was an abrupt reversal of the responses of the simple leaves to green and red light, accompanied by a reactivation of development under red light.

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THE MECHANISM OF AUXIN ACTION[1]

BARRY COMMONER AND DANIEL MAZIA

(WITH ONE FIGURE)

Plant growth hormones are known to produce a number of striking effects on various processes including cell enlargement, root initiation, bud inhibition, etc. It has also been shown that the auxins, and indole-3-acetic acid in particular, participate in some general protoplasmic processes. Support for this view has been most recently afforded in the demonstration by COMMONER and THIMANN (1) that this hormone is involved in part of the respiratory metabolism of plant cells.

The mechanism of the action of indole-3-acetic acid in the various growth processes which it influences is as yet unclear, however, even in the most favorable case of cell enlargement. Two theories concerning this auxin effect have been put forth. One suggests that the hormone lowers the resistance of the cell wall to turgor pressure, thereby permitting cell enlargement to take place. The other theory, suggested by CZAJA (2), holds that cell enlargement is the result of an increase in the intensity of an osmotic driving force. Although it has been the practice to consider these as two opposing theories it must be pointed out that both agree that the actual driving force in cell enlargement is osmotic in nature for it is clear that the turgor pressure is itself a consequence of osmotic forces.

No conclusive evidence in support of the wall-resistance theory has been presented thus far. The second theory has been objected to on the ground that growing plant cells do not show any increase in the osmotic pressure of their vacuole contents. The very failure of growing cells to exhibit a *reduction* in osmotic pressure while the vacuoles rapidly absorb water and increase in volume indicates, however, that the absolute solute content of the sap must be *increased* during this process.

Thus it becomes important to know whether indole-3-acetic acid influences the solute content of the cell sap and so by momentarily increasing the osmotic pressure, affects water uptake and cell enlargement. It would be expected that no observable increase in osmotic pressure would occur, since the uptake of water would counteract the increase in solute content. Increase in sap solute might result from the hydrolysis of stored material such as protein and starch or from the absorption of salts. Attention toward the latter alternative was drawn by the observation of THIMANN and SCHNEIDER

[1] This work was supported by grants received by one of the authors (B. C.) from the Penrose Fund of the American Philosophical Society (grant no. 500) and from the American Academy of Arts and Sciences.

(5), that the stimulation of the growth of Avena coleoptile sections by auxin is greatly enhanced by the presence of KCl in the medium.

The experiment reported here was designed to determine the effect of auxin on the salt absorption process in potato tuber slices, in order to learn whether the hormone may increase the sap solute content by stimulating this activity. Potato slices were prepared according to the method of STEWARD (3) and vigorously aerated for a period of eight days in solutions containing 0.001 M KCl and various concentrations of indole-3-acetic acid. Salt absorption was followed by determining the electrical conductivity of the solutions and wet weight was determined in a highly reproducible manner by weighing the tissues after blotting twice.

Since the slices were not previously rinsed they all went through the expected period of salt loss during the first 24 hours and then began to absorb salt actively. During this time all the slices absorbed water, since the external solution was hypotonic, but the relative amount absorbed in the different auxin concentrations followed a curve with an optimum at about 10 mg. per liter.

Figure 1 represents the data for the two-day period following the initial day of salt loss. The upper curve represents the amount of salt absorbed during this 48-hour period; the lower curve indicates the corresponding changes in wet weight during that time. It is obvious that these processes are affected by the concentration of auxin to which the slices are exposed. The parallelism between the two curves is clear and there is a definite maximum in both salt absorption and water uptake rates at a concentration of 10–20 mg. of auxin per liter. It should be noted that in the non-maximal auxin concentrations there is some salt absorbed while water is lost. The inverse relation holds for these concentrations only during this period of the time-course of the experiment, and when the changes during the total eight-day period are calculated there is a close correspondence between the positive absorption of both salt and water. An experiment similar to the one cited above but involving large numbers of Avena coleoptile sections gave the same results.

These data indicate that the salt absorption mechanism of these plant cells is affected by auxin in a manner which parallels the hormone's influence on cell enlargement, for the function relating auxin concentration to growth always follows a curve of the type shown in figure 1. Furthermore, the data indicate that the absorption of salt is at least in part the driving force for the uptake of water and the consequent cell enlargement. This conclusion is also supported by the observation that potato slices placed in hypertonic sucrose solutions (0.2 M) and treated with KCl and a maximal concentration of indole-3-acetic acid actually gain water while slices which are not supplied with auxin lose water and become limp. This evidence contradicts the

"wall-softening" theory of auxin action, for it shows that auxin can exert its effect even when the cells are flaccid and no turgor pressure exists. It is strongly indicated, therefore, that the effect of auxin on water uptake and cell enlargement is a direct consequence of its effect on the salt absorption process.

These conclusions necessitate a careful consideration of the connection between the available information on salt absorption and cell elongation in plant cells. The work of STEWARD, HOAGLAND *et al.* (**4**) has already shown

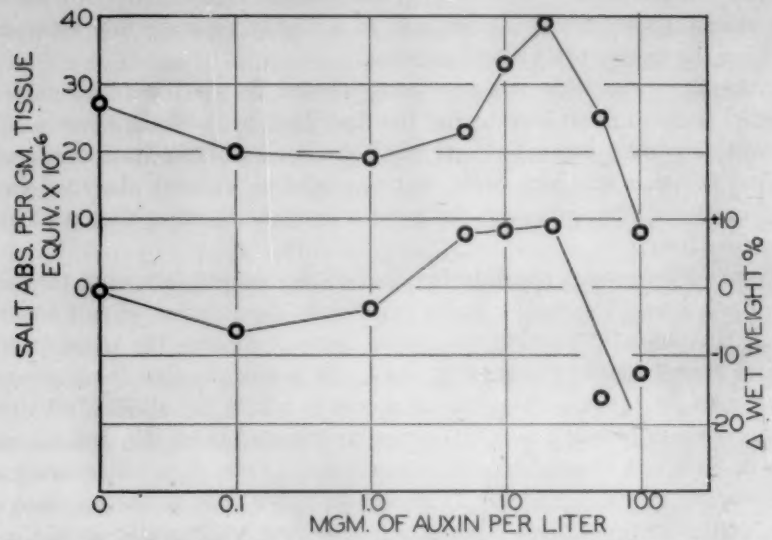

FIG. 1. The effect of various concentrations of indole-3-acetic acid on the amount of salt absorbed and the change in wet weight of potato slices during a 48-hour period.

that salt absorption depends on the activity of the cellular respiratory processes and will occur only under aerobic conditions. The metabolic changes which seem to be related to salt absorption involve, among other substances, the four-carbon dicarboxylic acids. These workers also report that salt absorption is always accompanied by an increase in cell size, and indicate that only those tissues which are capable of growth are able to absorb salt. This information bears a significant relation to the findings of COMMONER and THIMANN (**1**) on the relation between growth and respiration in the Avena coleoptile. Their data showed that the effect of auxin on cell enlargement is related to a parallel influence on the activity of that part of the cell respiration which involves the four-carbon dicarboxylic acid hydrogen transport systems. The similarity of the respiratory relations of both sets of phenomena is very apparent and indeed it seems likely that both salt absorption and cell enlargement depend on the same source of energy. Further sup-

porting evidence can be found in the fact that certain succulents such as the Crassulaceae are noted for the predominance of their four-carbon acid metabolism as well as for their water-binding powers.

It seems likely therefore that the effect of auxin on cell enlargement is a consequence of its effect on salt absorption and the water uptake which inevitably accompanies such an increase in sap solute. Auxin stimulates the enlargement of cells by augmenting the intensity of the osmotic driving force. The dependence of cell enlargement on respiratory processes may be viewed as a consequence of the respiratory dependence of the salt absorption process, and the evidence points to the four-carbon acid metabolism as the respiratory agent particularly related to these phenomena. A further report on the interrelations of these factors will appear soon.

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BRIEF PAPERS

ENZYMES AND THE LAW OF MASS ACTION

H. C. Eyster

It is known that the concentration of both the substrate and the end product influence enzymatic activity in a way that has been explained by the law of mass action.

Solutes other than substrate and end product have recently been found to have a retarding influence on enzyme action. For example, separately, potassium nitrate and cane sugar markedly reduce the rate at which soluble starch is digested to glucose by diastase. In the experimental investigation described in table I, 9 ml. of 1 per cent. soluble starch and 1 ml. of 1 per cent. diastase were used. The control had an additional 10 ml. of distilled water. The experimental mixtures had 10 ml. of 2 M commercial glucose, 10 ml. of 2 M cane sugar, and 10 ml. of 2 M potassium nitrate, respectively, in addition to the 9 ml. of 1 per cent. soluble starch and 1 ml. of 1 per cent. diastase. It can be readily ascertained that the commercial glucose, cane sugar, and potassium nitrate, respectively, had a final concentration of 1 M. As can be noted from the data in table I, 1 M cane sugar had slightly more retarding influence on diastase than did an equal concentration of commercial glucose, and 1 M potassium nitrate had twice as much retarding influence. The experiments were carried out at room temperature (about 25° C.).

TABLE I

Influence of solutes on the rate of digestion of soluble starch by diastase*

Duration of time	Control	Commercial glucose 1 M	Commercial cane sugar 1 M	Potassium nitrate C.P. 1 M
min.				
5	Blue	Blue	Blue	Blue
10	Lt. red	Dark red	Blue	Blue
15	Colorless	Dark red	Very dark red	Blue
20	Colorless	Red	Dark red	Blue
25	.....	Very light red	Red	Blue
30	.....	Colorless	Light red	Dark red
35	.....	Colorless	Colorless	Dark red
40	.....	.....	Colorless	Red
45	.....	.....	.....	Red
50	.....	.....	.....	Light red
55	.....	.....	.....	Light red
60	.....	.....	.....	Colorless

* The results presented are color reactions of a small aliquot portion with a drop of a dilute solution of iodine potassium iodide.

In order to throw more light on the problem, the effect of commercial glucose, cane sugar, and potassium nitrate on the adsorption of methylene blue by finely ground (about 80 mesh) activated charcoal was determined. The experiments were carried out at room temperature (about 25° C.) and had as their immediate object the determination of the maximum amount of a 1 per cent. aqueous solution of methylene blue one gram of the finely ground activated charcoal could adsorb in ten minutes in the presence of various concentrations of glucose, cane sugar, and potassium nitrate, respectively. Table II presents the data, which clearly manifest that solutes reduce adsorption on the charcoal particles.

TABLE II

INFLUENCE OF SOLUTES ON THE RATE OF ADSORPTION OF METHYLENE BLUE BY FINELY GROUND ACTIVATED CHARCOAL*

SOLUTES	0 M (CONTROL)	0.1 M	0.5 M	1 M
Commercial glucose	8 ml.	6–6.5 ml.	4.5–5 ml.	3–3.5 ml.
Commercial cane sugar	8 ml.	6.5–7 ml.	4–4.5 ml.	2–2.5 ml.
Potassium nitrate (C.P.)	8 ml.	7.5–8 ml.	5–5.5 ml.	4–4.5 ml.

* The results presented are threshold values—maximum amount of methylene blue (1 per cent. aqueous solution) capable of being completely adsorbed in 10 minutes. Total volume in each case was 100 ml.

For the control, 8 ml. 1 per cent. aqueous methylene blue, 1 gram activated charcoal, and 92 ml. of distilled water were used. Eight ml. had been found to be threshold value for control in which there were no solutes. The 0.1 M trials had threshold value (t) of 1 per cent. aqueous methylene blue, 5 ml. of 2 M solute (glucose, cane sugar, potassium nitrate), 1 gram activated charcoal, and (95-t) ml. of distilled water—the total volume less the charcoal was 100 ml. The threshold value (t) of 1 per cent. aqueous methylene blue, 25 ml. of 2 M solute (glucose, cane sugar, potassium nitrate), 1 gram activated charcoal, and (75-t) ml. of distilled water were used for 0.5 M concentrations. Here, too, the total volume of the solution, less the activated charcoal, was 100 ml. For the 1 M concentrations the threshold value (t) of 1 per cent. methylene blue, 50 ml. of 2 M solute (glucose, cane sugar, potassium nitrate), 1 gram activated charcoal, and (50-t) ml. of distilled water were used. As in the preceding trials, here too, the total volume without the activated charcoal was 100 ml. The methylene blue solution, solute solution and distilled water were thoroughly mixed. The 1 gram of activated charcoal was added with an approximately standard amount of agitation. At the end of ten minutes the mixture was filtered to determine whether all of the methylene blue had been adsorbed.

The data on the two sets of experiments present evidence which indicate

that the solutes interfere in some way both with enzyme activity and with adsorption. EYSTER (1) has recently found that enzyme activity is fundamentally adsorptive, because he was able to duplicate with charcoal and methylene blue, the luciferase experiments described by JOHNSON, BROWN, and MARSLAND (2). The adsorptive mechanism of enzyme action is influenced by solutes probably in one or both of two ways. First, solutes may reduce the actual adsorptive capacity of the enzyme for the substrate, by themselves being adsorbed by the enzyme; and secondly, the solutes may reduce the mobility of the substrate molecules, thereby decreasing the rate at which the substrate can be adsorbed. While chemical mass action may have some influence in affecting the rate of enzyme activity, it is more likely that solutes, regardless of whether they are substrate substances and products, exert most of their influence on enzymatic activity by reducing the adsorptive capacity of the enzyme for the substrate.

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VERMILLION, SOUTH DAKOTA

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AERIAL ROOTS FROM OLD TREE WOUND[1]

WILLIAM A. NESBITT

(WITH ONE FIGURE)

Recently, a northern red oak tree was found on the Bent Creek Experimental Forest, Buncombe County, North Carolina, with an unusual and interesting group of aerial roots originating from the callused area surrounding an old wound.

This tree, shown in the accompanying photograph, is about 100 feet high, has approximately 3 log lengths and is 33.5 inches in diameter at 4.5 feet above the ground. It is an overmature or decadent tree, with a rather flat top, two or three large dead limbs, and a large "conk" on the bole about 40 feet above ground. Otherwise it is apparently in good health. According to ring counts on the callused area surrounding the wound, 11 years have elapsed since some undetermined cause killed approximately one-third of the circumference, from the ground line to an average height of 7 feet. Most of the bark over the wounded or dead area was intact when the tree was discovered a short time ago. Approximately two inches of the sapwood has decayed and is now in a dry powdery-rot condition, with hundreds of small worm holes extending into the heartwood. The area surrounding the wound has callused in the usual manner. The live portion directly above the wound, and at one point midway up on the side of the callused area, has sent out groups of rope-like growths extending to the ground and forming fibrous roots. There are three separate such growths, approximately one inch in diameter, reaching the ground, with several smaller ones extending varying distances down through the rotted sapwood under the bark. A portion of the fibrous roots from one of the strands is shown lying on top of the hammer in the photograph on page 690. These rope-like aerial roots are provided with a bark covering similar to ordinary roots, and are independent of the parent tree, except at their point of origin.

APPALACHIAN FOREST EXPERIMENT STATION
ASHEVILLE, NORTH CAROLINA

[1] Contribution from the Appalachian Forest Experiment Station.

FIG. 1. Red oak tree with aerial roots which have arisen from the callus of a wound.

NOTES

Nineteenth Annual Meeting.—The nineteenth annual meeting of the American Society of Plant Physiologists is scheduled to be held in New York City from December 29 to 31, 1942. The headquarters of the society will be the Commodore Hotel, Lexington Ave. and 42nd St. The annual dinner will again be the scene of the awards, the eighth STEPHEN HALES, and the nineteenth CHARLES REID BARNES award. It is hoped that everyone who attends the meetings will try to be present for these social functions. Immediate reservation of tickets for the dinner is usually necessary on arrival.

Some symposia are to be presented, and a session with relation to the war is being planned. It seems probable that attendance will be sharply reduced because of the transportation conditions. At the same time, scientific work on long-time problems should continue, or there will be a very serious time loss in the progress of science. This must not be allowed to happen, if such a calamity can be averted. New York is close to a very large number of scientists, so that the meetings should still have a good attendance, and many valuable contributions.

Western Section.—In the absence of any direct report from the secretary of the Western Section meeting of last June, the editor refers members to the account published in SCIENCE n.s. **96**: 146–147. 1942. The officers of the section during 1942–1943 are: chairman, E. T. BARTHOLOMEW; vice-chairman, F. J. VEIHMEYER; and secretary, D. I. ARNON.

Life Membership Committee.—The committee in charge of the selection of a candidate for the nineteenth award of the CHARLES REID BARNES life membership is constituted as follows: Chairman, WILLIAM E. SEIFRIZ; GEORGE O. BURR, H. C. SAMPSON, G. W. SCARTH, and J. C. GILMAN. It is never an easy task to make this selection, but it is a privilege to share in making it. These members may be relied upon to make a very careful and worthy choice. The announcement of their selection will be a part of the dinner festivities in New York.

Stephen Hales Committee.—The candidate for the HALES award is selected by the last three recipients of the award, of whom the first of the three to receive the award is chairman. Dr. KENNETH V. THIMANN of Harvard University is chairman of the 1942 committee, and he is assisted by Dr. JOHN W. SHIVE, of the New Jersey Agricultural Experiment Station, and Dr. PHILIP R. WHITE, of the Rockefeller Institute for Medical Research, Princeton, New Jersey. The award will likewise be announced at the annual dinner.

Errata.—Very few errata have been reported from volume 17. One has been sent in by Dr. R. COLLANDER for his paper in volume 16. As this changes the meaning of his summary, members are urged to correct the error *in situ*. It was unfortunate that the proofs could not be submitted to Dr. COLLANDER on account of war conditions. He had no opportunity to see the proofs personally. All authors are requested to report any errors found. The editors do not have time to hunt for them, and would appreciate each author's help. The errata are listed at the end of the table of contents in this number of PLANT PHYSIOLOGY.

Inaugural Representative.—The American Society of Plant Physiologists was represented by Dr. S. F. TRELEASE, Columbia University, at the inauguration on September 30, 1942, of HARRY NOBLE WRIGHT as president of New York University.

Memorial Committee.—The memorial committee has received much attention from president LOOMIS, and the committee has been reconstituted. The chairman is Dr. R. P. HIBBARD, Michigan State College; and Drs. J. S. CALDWELL, U.S.D.A.; R. B. HARVEY, University of Minnesota; and S. F. TRELEASE, Columbia University. This committee, as part of its duties, is charged with keeping in mind all occasions when memorial meetings would be appropriate, such as, for instance the 100th anniversary of the death of DE SAUSSURE, the 100th anniversary of the word "protoplasm," etc., occasions that should not be allowed to slip by without some remembrance of the great leaders of the past.

Training of Science Teachers.—The Cooperative Committee on Science Teaching, consisting of B. S. HOPKINS and MARTIN V. MCGILL representing the American Chemical Society, K. LARK-HOROVITZ and GLEN W. WARNER representing the American Association of Physics Teachers, G. P. CAHOON and ROBERT J. HAVIGHURST representing the National Association for Research in Science Teaching, A. A. BENNETT and RALEIGH SCHORLING representing the Mathematical Association of America, and OSCAR RIDDLE and WALTER F. LOEHWING representing the Union of American Biological Societies, has prepared a preliminary report embodying the recommendations concerning teacher training and certification of high school science teachers.

A national survey indicates that the more serious problems are found in small high schools with five or less teachers called upon to offer a curriculum of 12 to 20 subjects in such a way that each teacher is obliged to instruct in three or four subjects. College training for this type of high school teacher is generally too narrow in so far as it does not readily permit training in at least two or three fields of science. From the standpoint

of teacher training the problem centers chiefly in Liberal Arts Colleges on account of the fact that approximately half of the teachers in public high schools are liberal arts graduates. The actual number of such teachers in service is large enough to justify recognition of this problem in formulation of liberal arts curricula in American Universities.

In reference to training of science teachers the Committee recommends (1) that approximately one-half of the four-year college program be devoted to courses in science as a combination of any three of the following subjects: (a) biological science (including both botany and zoology), (b) chemistry, (c) earth science, (d) mathematics, (e) physics. Science departments in the colleges are urged to cooperate among themselves and with the department of education in devising such a program of teacher preparation.

In reference to licensing of teachers, the Committee recommends specific certification in three subjects to correspond to the above program for teacher-training institutions, with the following requirements for certification; namely, a total of at least 60 semester hours' credit in science, comprising a minimum of 18 hours' credit in each subject for which the certificate is granted, except that 24 hours' credit should be the minimum for certification in biology, including courses in both botany and zoology. For certification to teach general science in the junior high school only, the Committee recommends as an alternative to certification in three subjects the requirement of a minimum of 15 hours in biological science, 15 hours in physical science (including both physics and chemistry), 6 hours in earth science, and 6 hours in astronomy. These are recommended as minimal requirements for the teaching certificate though the Committee favors a five-year training period for teachers as a professional degree. It does not, however, seem appropriate to recommend that an additional year be added to the undergraduate course at the present time.

With reference to certification of science teachers, the Committee recommends abolishing the practice of awarding certificates in the whole field of science on the basis of a minimum of 15 hours as is currently practised in many localities. The problem of certification would be simplified if the number of teaching combinations could be reduced. If teaching combinations could be stardardized, the entire matter of teacher training could be simplified because the prospective teacher could then be sure of using certain science combinations upon graduation.

The practical results of this report will depend upon what is actually done in colleges and in state departments of education about the problems of preparation and certification of teachers. There are two ways by which scientists and educators can work to influence practices in colleges and state departments of education.

In colleges, members of the department most interested in these problems

can act on their own initiative to broaden the science preparation of prospective high school teachers. Much progress has already been made by this method, as the answers to our questionnaire show. College science departments may also cooperate with departments of education to devise professional programs for the preparation of science teachers.

The best method of effecting changes in certification procedures is through organizing committees on state and local bases which can make their voices heard in the state capitol and the state university. This method has been effective since 1939 in the State of Indiana, where a statewide committee has made proposals for certification which are being considered by the State Department of Education. In Pennsylvania there is a Committee for the promotion of science in secondary education for the State of Pennsylvania. This Committee recently issued a report on certification of science teachers in secondary schools which recommends raising the standards for certification in that state.

The Cooperative Committee on Science Teaching stands ready to advise any state or local group which is interested in the problems treated in this report. Correspondence is solicited. A copy of the Preliminary Report of the Cooperative Committee on Science Teaching will be sent free to anyone who writes for it to the Chairman, ROBERT J. HAVIGHURST, The University of Chicago.

Necrology.—It is a regret to have to announce the death of a number of our members. Some of the deaths occurred many months back, but were not reported to the editorial office, so no obituaries have been provided. In some cases it has not been possible to obtain data regarding the men. Such as have been cleared up are presented. Others will be printed later, if appropriate data can be obtained.

Elmer Severn Miller.—The death of this brilliant young biochemist and plant physiologist is a great loss to science. It may be recalled that he was a specialist in the field of biological spectroscopy, and that he published in 1939 an important book, *Quantitative Biological Spectroscopy*, from the Burgess Publishing Co., of Minneapolis.

Dr. MILLER was born at Thief River Falls, Minnesota, June 18, 1905. After graduation from high school, he came to the University of Minnesota, where he received a B.S. degree in biochemistry in 1930. The following summer he did field work for the U. S. Department of Agriculture in the western deserts and became interested in plant physiology. It was in this subject that he did his thesis for the Ph.D., which he received, also at Minnesota, in 1932.

On finishing his work for the doctorate, he was appointed to a National

Research Council fellowship, which he held at the University of Chicago. After two years on this appointment, he became research associate in chemistry at Chicago. In 1935 he returned to Minnesota as instructor in plant physiology. He was soon appointed to an assistant professorship.

During his student days he became interested in photosynthesis and other biological effects of light. This remained a major interest both in teaching and research. His many publications are well known. He took an active part in national conferences on applied spectroscopy and was a member of numerous scientific societies. Through his tireless efforts important cooperative researches in agriculture and medicine were being maintained. His death on June 11, 1941, was a great professional loss not only to the University of Minnesota, but to his colleagues and friends throughout the country. To those who knew him, it is also a deep personal loss.

Ondess Lamar Inman.—On July 21, 1942, Dr. O. L. INMAN died at Mt. Sinai Hospital in New York City, where he had gone for treatment after he had been taken ill with a streptococcus infection which got into the blood stream. He went to New York early in February, after unsuccessful attempts to head it off in late January. His wife, FRESSA BAKER INMAN, Director of Admissions at Antioch College, went with him to New York.

He was born at Bloomfield, Indiana, November 11, 1890. His degrees were obtained at Indiana University (A.B., 1915), the University of Idaho (B.S., 1916), and at Harvard University (Ph.D., 1921). He was a teaching instructor in botany at Idaho for three years, and Austin Teaching Fellow at Harvard in 1919–1920. He became associated with the Department of Biology at Antioch College in 1921, immediately upon completion of his work at Harvard. He served as dean at Antioch in 1929–1930, but in 1930 became director of the C. F. Kettering Foundation for the Study of Chlorophyll and Photosynthesis. This was one of the important research projects established at Antioch under the presidency of Dr. ARTHUR E. MORGAN. Under Dr. INMAN's leadership, the project was carried on with distinction, and many papers were published by him, his coworkers and assistants. He was a member of many scientific organizations, and served as vice president of the Ohio Academy of Science in 1935. A memorial service was held in Kelly Hall, at Antioch College, on July 23, at which fine tributes were paid to him by Dr. MORGAN and others. His loss will be keenly felt.

Lee Blanton Nash.—On October 21, 1941, LEE BLANTON NASH, of the University of Florida, died as the result of complications following a rupture of the appendix. He was born near Tipton, Indiana, July 4, 1914. He received the B.S. degree at Purdue University in 1935, having majored in agricultural chemistry. He was an assistant in the State Chemist's labora-

tory at Purdue University from 1934 to 1936, working with Dr. H. R. KRAYBILL. He went to Cornell University in 1936, where he remained until 1940. He was assistant in vegetable crops at Cornell, mainly engaged in analyses of vegetables. He wrote a number of papers during this period, usually in collaboration with Prof. ORA SMITH, on the cooking quality of potatoes as influenced by light, mineral nutrition, irrigation, etc.

He was appointed assistant in horticulture at Florida in March, 1941. At this time he married JOSEPHINE CODER, of South Williamsport, Pennsylvania, who was a gifted musician. His modest and unassuming manner, and pleasing personality endeared him to his colleagues, and had won for him a wide circle of friends. He was capable and zealous in his research, and had earned the respect of everyone who knew him.

Laurence Sorver Moyer.—The first war casualty, so far as we know, among our membership, was the loss of Dr. LAURENCE S. MOYER on June 8, 1942, in the collision of two blimps off the coast of New Jersey. He was a native of Norristown, Pennsylvania, where he was born on July 4, 1907. He had earned his degrees at the University of Pennsylvania (A.B., 1929; Ph.D., 1933). He was assistant botanist at Pennsylvania University from '27 to '29, and assistant instructor from '29 to '33. He was appointed to a National Research Council fellowship which he enjoyed for two years (1933–1935), at the University of Minnesota in agricultural biochemistry. The following year he was Sterling fellow in Public Health at Yale, and was then called to Minnesota as instructor in botany in 1936. His main interests in research were in the field of cellular physiology, electrokinetics of latex, electrophoretic properties of proteins, sterols, bacteria, and wetting properties of colloids. A brief obituary by his former teacher and close friend, Dr. WILLIAM SEIFRIZ, appears in SCIENCE n.s. **96**: 103–104. 1942.

Robert Eugene Oltman.—At the age of 33 years Dr. ROBERT E. OLTMAN, chief chemist of the Minnesota Valley Canning Co., died suddenly on July 24, 1942, of pulmonary embolism. His passing removed from the canning industry one of its most brilliant young scientists. Dr. OLTMAN's researches in the fields of plant physiology and chemistry as pertaining to the canning industry have yielded noteworthy results. During the past several years he has been engaged in developing an outstanding research laboratory at Le Sueur, Minnesota, where he had his headquarters. His work led him deeply into the field of plant chemistry in an attempt to improve and coordinate many processes involved in the production and preservation of high-quality vegetables.

Dr. OLTMAN was born in Cleveland, Ohio, Dec. 5, 1908, and was educated in the public schools of Cleveland. Upon graduation from High School in

1927 he entered the service of the U. S. Department of Agriculture in its campaign for the eradication of the corn borer, and during the next four summers became successively Inspector, Field Scout Foreman, Assistant Field Supervisor, and Field Supervisor, with large crews of men under his supervision.

During this time, and after a year at Western Reserve University, he entered Oberlin College from which he was graduated in 1932. There he showed an unusual capacity for research and invention. During his Junior and Senior years he worked out a method for the quantitative determination of pigments, that culminated in his invention of a photoelectric colorimeter for the measurement of pigment concentration, to replace the usual visual method. This instrument he exhibited and described at the general session of the Ohio Academy of Sciences in April, 1932, with a paper entitled *A New Instrument for the Quantitative Determination of Chlorophyll.* At the same time he was working on the effect of different wave lengths of ultra violet light on leaf pigments, by means of new types of cellophane filters. Some of his results were presented at the same meeting of the Ohio Academy in a paper on *The Effect of Ultra Violet Light of Various Wave Lengths on the Development of Chlorophyll in Seedlings.*

After graduation from Oberlin Mr. OLTMAN entered the University of Minnesota as a graduate student and Assistant (later Instructor) in Plant Physiology, receiving his Ph.D. degree in 1936.

He then joined the research department of the Minnesota Canning Company for a year, after which he was for two years Director of Research for Fine Foods of Canada, Ltd., Toronto, where his work was largely administrative and organizational. Returning to the research department of the Minnesota Canning Company he was made Chief Chemist, although the research which he carried on and directed ranged widely over the methods of growing, harvesting, processing, packing, and shipping of their products. He was only on the threshold of his scientific career when he was cut off.

Dr. OLTMAN was a member of the A.A.A.S. and of the American Chemical Society, and a Fellow of the Canadian Institute of Chemistry. He leaves a wife and son, his parents, and one sister.

Chemistry of Insecticides and Fungicides.—The progress in development of insecticides and fungicides has been very rapid in recent years, and we now have a timely volume devoted to the chemistry of these useful control agents. The author is Dr. DONALD E. H. FREAR of The Pennsylvania State College, and the publishers are D. Van Nostrand Co., Inc., 250 Fourth Ave., New York. The introduction briefly discusses man versus pests, methods of control, and types of insects. The main body of the work falls into five parts, dealing with: I. Stomach poisons or protective insecticides, such

as arsenicals, fluorine compounds, hellebore, dinitro derivatives, phenothiazine, and miscellaneous organics; II. Contact poisons or eradicant insecticides, such as pyrethrum, nicotine, rotenone, deguelin, toxicarol, tephrosin, sumatrol, quassia, croton, organic sulphurs, amines, etc., inorganic sulphur compounds, oils, and many kinds of fumigants; III. Fungicides, such as the copper compounds, mercury compounds, zinc compounds, formaldehyde and other fungicides, and the wood and cellulose preservatives; IV. Spray supplements and residue removal, with special attention to wetting, spreading, and emulsifying agents, tolerances, dry wiping, flotation washers, mechanical washers, solvents, etc.; and V. Analytical methods, both macro and micro methods being presented.

This is an excellent handbook for those in charge of plant production problems, where crops must be protected against loss from our insidious enemies, the insects and fungous diseases. It is very straightforward, not too technical, and ought to be a source of direct aid to all conservationists. It is obtainable from the publishers at $4.00 net. Fifteen chapters, 300 pages.

Deciduous Orchards.—A book with this title by Dr. WILLIAM HENRY CHANDLER of the Department of Horticulture, The University of California, comes from the press of Lea and Febiger, Philadelphia. The work lays emphasis upon the processes and responses of trees, and relationships, a framework upon which all knowledge about orchards can be hung. While there is some repetition involved in the presentation, these duplications are abundantly justified. Devoted to deciduous orchards, it is a well unified work, and it includes the amentiferous nuts and tung-oil trees in addition to the usual pome, stone, and aggregate fruits, also the persimmon, pawpaw, pistachio nut, etc.

The preface indicates that Dr. CHANDLER has learned much about students and their needs, as well as about orchard trees, and that he puts the knowledge of fundamentals first in orchard practice.

Nutrition occupies a prominent place in the presentation, chapter I presenting a general view of the needs of plants. Then come chapters on tree and variety, resting buds, flower bud formation, pollination and fruit setting, growing fruit, mature fruit, and killing temperatures. Attention is turned following these, to propagation, transplanting, soil relations, nutrients for trees, essential elements (and those which influence growth), water supply, pruning, and responses to these factors. The final chapters deal with particular groups of fruits, already mentioned. It is a very readable text, and provides students of horticulture with an admirable source book of fundamentals. It contains 438 pages, 109 figures, and is listed at $4.50 per copy.

High Polymers.—A series of monographs on high polymers is being published by Interscience Publishers, Inc., New York City. So far five volumes in this series has appeared. Volume IV is entitled *Natural and Synthetic High Polymers.* It was originally written in German by KURT H. MEYER, of Geneva. It is here translated into English by L. E. R. PICKEN, of Cambridge, England. So many biological substances are now known to be high polymers that a book of this kind is of very great value in understanding the chemical constitution of the natural synthetics. The larger sections of the work may be outlined thus: Introduction; the study of high polymers; inorganic high polymers; high-polymeric hydrocarbons and their derivatives; polymeric ethers, esters, sulfides, etc.; cellulose and its derivatives; substances related to or associated with cellulose; starch and related carbohydrates; the proteins; the properties of high polymers in solution; films, foils and membranes; and the molecular structure of animal and plant tissues. It is appropriately indexed, and has in an appendix curves for determining specific viscosity.

While this is a technical work, it is of very great importance to understand the manner in which polymers are built up. Much attention is given to rubber, for instance, natural and artificial; and such materials as lignin, pectins, gums and mucilages, hemicelluloses, starch, glycogen, proteins, enzymes, etc., are the daily problem of biochemist and plant physiologist. This excellent work will help the student to gain modern conceptions of plant chemistry, and of the forces which hold polymeric substances together in the living body. It is recommended for serious study.

It has 690 pages, and the publisher's price is $11.00 per copy. There are 180 text figures.

GENERAL INDEX[1]

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[1] Papers published in PLANT PHYSIOLOGY are indexed in *The Agricultural Index*, The W. H. WILSON Co., New York.

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